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Proton tautomerism and stereoisomerism in 1,3-thiazolidinone derivatives

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CONTENTS

ABSTRACT

The thesis presents the synthesis and structural analysis of a series of hybrid 1*H*-pyrazolin/thiazolidinones as well as of 5-ylidene derivatives of 4-phenylamino-1,3-thiazol-2(5*H*)-ones with the phenyl ring variously substituted with the -OH, -OCH_3, and -CF_3 groups. The aim of the structural analyses was to elucidate the structural changes and tautomeric effects in the amidine system caused by the various chemical modifications of the thiazolidinone core. The solid phase structures were characterized by single-crystal and powder X-ray diffraction, and by FT-IR spectroscopy. To examine the tautomeric effect as a possible dynamic phenomenon, the liquid phase (DMSO solution) behavior of theinvestigated compounds was studied by ^{1}H and ^{13}C NMR spectroscopy. As a major conclusion, the exceptional stability of the amino tautomeric form with synperiplanar conformation of all the title compounds has been established both in the solid and liquid phase. The structures were analyzed from the point of view of the electronic effects and conformational freedom of the molecules. The differences in electronic structure resulting from substituent swapping were also analyzed. In addition, the intermolecular interactions and supramolecular architecture of the crystal lattices were highlighted. Computational analyses (density functional, DFT; and atoms-in-molecules, AiM; theory) were carried out to theoretically explain the experimental data. The antiproliferative activity of selected compounds was evaluated and the most susceptible cancer cell lines (renal cancer) were identified.

STRESZCZENIE

W ramach pracy doktorskiej przeprowadzono syntezę oraz badania strukturalne i spektroskopowe serii nowych pochodnych C5-ylidenowych 4-fenyloamino-1,3-tiazol-2(5*H*)-onu o dużym znaczeniu farmakologicznym, zawierających podstawniki ylidenowe o odmiennych właściwościach elektronowych, to jest podstawnik dimetyloamino-metylidenowy o właściwościach elektrono-donorowych i podstawnik metoksykarbonylo-metylidenowy o właściwościach elektrono-akceptorowych, a także pochodnych 5-dimetylo-aminometylideno-4-fenyloamino-1,3-tiazol-2-onu z pierścieniem fenylowym podstawionym grupami -OH, -OCH_3 względnie -CF_3. Osobną podgrupę pochodnych 5-ylideno-4-fenylo-amino-1,3-tiazol-2(5*H*)-onu stanowiły 5-ylideno-4-(*p*-R-fenylo)amino-1,3-tiazol-2(5*H*)-ony otrzymane na drodze syntezy, której celem było wprowadzenie do cząsteczek wraz z podstawnikiem ylidenowym innych heterocykli. Badania zostały podjęte głównie w celu lepszego zrozumienia wpływu zmian strukturalnych i elektronowych na zjawisko tautomerii protonowej oraz stereoizomerii pochodnych 5-ylideno-4-fenyloamino-1,3-tiazol-2(5*H*)-onu. Struktury analizowanych związków zostały określone za pomocą spektroskopii ^{1}H i ^{13}C NMR i FT-IR oraz analizy rentgenograficznej. Przeprowadzono również obliczenia teoretyczne potwierdzające obserwacje eksperymentalne. Uzyskane wyniki badań odnoszących się do zjawiska aminowo-iminowej tautomerii protonowej w grupie pochodnych 5-ylideno-4-fenyloamino-1,3-tiazol-2(5*H*)-onu wyjawiły wyjątkowo dużą trwałość formy tautomerycznej aminowej oraz brak wpływu na zjawisko aminowo-iminowej tautomerii protonowej obecnych w pierścieniu fenylowym podstawników zarówno o charakterze elektrono-donorowym (-OH, -OCH_3), jak i o charakterze silnie elektrono-akceptorowym (CF_3). Wykazano pojawienie się znaczących efektów rezonansowych w obrębie grupy amidynowej i podstawnika ylidynowego o charakterze elektrono-donorowym, wywierających wpływ na kształt i właściwości cząsteczek. Jednocześnie stwierdzono, że reszta fenyloaminowa ustawia się w cząsteczkach badanych związków synperiplanarnie zarówno w ciele stałym, jak i w roztworze (DMSO), zaś podstawnik ylidenowy przyjmuje konfigurację *Z*.

LIST OF ORIGINAL PUBLICATIONS THAT ARE THE SUBJECT OF THE DISSERTATION

1. **Pyrih, A.**, Jaskolski, M., Gzella, A. K. & Lesyk R. (2021). Synthesis, structure and evaluation of anticancer activity of 4-amino-1,3-thiazolinone/pyrazoline hybrids. *J. Mol. Struct.* **1224**, 129059; IF = 3.800, MEiN = 70.

2. **Pyrih, A.**, Łapiński, A., Zięba, S., Lesyk, R., Jaskolski, M. & Gzella A. K. (2023). Proton tautomerism and stereoisomerism of 4-amino-1, 3-thiazol-2(5*H*)-one derivatives bearing substituents with opposite electronic effects: Synthesis, structure and spectroscopic studies. *J. Mol. Struct.* **1274**, 134441; IF = 3.800, MEiN = 70.

3. **Pyrih, A.**, Łapiński, A., Zięba, S., Mizera, A., Lesyk R., Jaskolski, M. & Gzella, A. K. (2023). Proton tautomerism in 5-dimethylaminomethylidene-4-(*o*-,*m*-,*p*-hydroxyphenyl)-amino-1,3-thiazol-2(5*H*)-ones: synthesis, crystal structure and spectroscopic studies. *Acta Cryst.* B**79**, 220–232; IF = 1.900, MEiN = 140.

4. **Pyrih, A.**, Łapiński, A., Zięba, S., Mizera, A., Lesyk R., Gzella, A. K. & Jaskolski, M. (2023). Proton tautomerism and stereoisomerism in 5-[(dimethylamino)methylidene]-4-[3/4-(trifluoromethylphenyl)amino]-1,3-thiazol-2(5*H*)-ones: synthesis, crystal structure and spectroscopic studies. *Acta Cryst.* C**79**, 480–490; IF = 0.800, MEiN = 140.

5. **Pyrih, A.,** Łapiński, A, Zięba, S., Mizera, A., Lesyk, R., Gzella, A. K. & Jaskolski M. (2024). Proton tautomerism and stereoisomerism in isomeric 4-(methoxyphenyl) amino-1,3-thiazol-2(5*H*)-one derivatives: Synthesis, crystal structure and spectroscopic studies. *J. Mol. Struct.* **1295**, 136748; IF = 3.800, MEiN = 70.

THE RESULTS OF THE PROJECT WERE PRESENTED AT THE FOLLOWING SCIENTIFIC CONFERENCES

- **Pyrih, A.**, Gzella, A. K., Lesyk, R., Jaskolski M. Conformational polymorphism of 4-(3-methoxyphenyl)amino-2-thiazolinone. Lviv Chemical Readings - 2019, Lviv, Ukraine, 2–5 June 2019, poster *O*33.

- **Pyrih A.**, Gzella A., Lesyk R., Jaskolski M. Amine-imine proton tautomerism in isomerism 2-phenylamino-1,3-thiazol-4(5*H*)- and 4-phenylamino-1,3-thiazol-2(5*H*)-one derivatives. 32nd European Crystallographic Meeting, Vienna, Austria, 18–23 August 2019, poster *MS*41-*P*03.

- **Pyrih, A.**, Gzella, A. K., Lesyk, R., Jaskolski M. Amine-imine tautomerism in isomeric 2-(*o*-,*m*-,*p*-hydroxyphenyl)-amino-1,3-thiazol-4(5H)-ones. Joint Polish-German Crystallographic Meeting. Wrocław, Poland, 24–27 February 2020, p. 37, poster *P*98.

- **Pyrih A.**, Jaskolski M., Lesyk R., Gzella A. On the selectivity of methylation of the amidine system and stereoisomerism of 3-alkylated derivatives of 5-methoxycarbonylmethylidene-4-phenylimino-1,3-thiazol-2(5*H*)-one. 33rd European Crystallographic Meeting, Paris, France, 23–27 August 2022, poster *MS*28-1-12.

LIST OF OTHER SCIENTIFIC ACHIEVEMENTS

➢ List of original publications

1. **Pyrih A.**, Berninger M., Gzella A., Lesyk R., Holzgrabe U. (2018). Synthesis and evaluation of antitrypanosomal activity of some thiosemicarbazide derivatives of 1-butyl-6-fluoro-7-morpholino-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid. *Synth. Commun.* **48**, 1883–1891; IF = 1.439, MEiN = 20.
2. Kaminskyy D., Subtel'na I., **Pyrih A.**, Shtoyko D., Susel A., Gzella A., Lesyk R. (2017). One-Pot Synthesis of 5-Ene-4-aminothiazol-2(5*H*)-ones and Chromeno[2,3-*d*]thiazol-2-ones. *Synlett* **28**, 811–814; IF = 2.369, MEiN = 25.

➢ Active participation in scientific conferences

1. **Pyrih A.**, Lesyk R., Gzella A.
   Structure of 4-(*p*-halogenophenyl)amino-5-ylideno-2-thiazolidones in solid state.
   60 Konwersatorium Krystalograficzne (Polish Crystallographic Meeting), Wroclaw 28–30 czerwca 2018, poster *B*21.
2. Suseł A., Łapiński A., **Pyrih A.**, Lesyk R., Gzella A.
   Structural studies of 4-(*m*/*p*-trifluorophenyl)amino-2-thiazolidones in solid and liquid phases.
   60 Konwersatorium Krystalograficzne (Polish Crystallographic Meeting), Wrocław 28–30 czerwca 2018, poster *A*14.
3. Suseł A., Łapiński A., **Pyrih A.**, Lesyk R., Gzella A.
   Structural studies of 4-(*p*-halogenophenyl)amino-2-thiazolidones in solid and liquid phases
   60 Konwersatorium Krystalograficzne (Polish Crystallographic Meeting), Wrocław 28–30 czerwca 2018, poster *A*13.
4. Suseł A., Łapiński A., **Pyrih A.**, Lesyk R., Gzella A.
   Studies on proton amine-imine tautomerism phenomenon of isomeric 4-methoxyphenyl-amino-2-thiazolidones.
   60 Konwersatorium Krystalograficzne (Polish Crystallographic Meeting), Wrocław 28–30 czerwca 2018, poster *A*12.
5. **Pyrih A.**, Lesyk R., Gzella A.
   X-ray analysis of amine-imine proton tautomerism in a group of 4-phenylamino-2-thiazolinone derivatives in a solid state
   59 Konwersatorium Krystalograficzne (Polish Crystallographic Meeting), Wroclaw 28–30 czerwca 2017, p. 222, poster *B*36.

6. **Pyrih A.**, Lesyk R., Gzella A.
   X-ray characterization of amine-imine proton tautomerism in the group of 4-phenylamino-2-thiazolines in the solid state.
   XVI Lviv Chemical Readings – 2017, Lviv, Ukraine 28–31 May 2017, Book of Abstracts, poster *O*-19.
7. Suseł A., **Pyrih A.**, Subtelna I., Lesyk R., Gzella A.
   Structure of 4-amino-1,3-thiazol-2(5*H*)-one and 4-phenylamino-1,3-thiazolidyn-2-one in solid and liquid phases – results of X-ray structutral and spectroscopic analysis.
   58 Konwersatorium Krystalograficzne (Polish Crystallographic Meeting), Wrocław 22–24 czerwca 2016, p. 239–240, poster *B*45.
8. **Pyrih A.**, Kaminskyy D., den Hartog G., Golota S., Bast A., Lesyk R.
   Antioxidant activity of some 4-thiazolidinone derivatives IX International meeting “From Molecular to Cellular Events in Human Pathologies”.
   Lviv, Ukraine, 19–22 September 2016, Biopolimers & Cell 2016. Vol. 32. N 4. *pp.* 300–324, poster.

1. INTRODUCTION

2-Aminothiazolidinones

The vast majority of new medicines approved annually by the Food and Drug Administration (FDA) are small molecules, and the overwhelming majority of them are nitrogen-containing heterocycles [de la Torre *et al.*, 2023]. 4-Thiazolidinones are one of such so-called privileged nitrogen- and sulfur-based heterocycles, which have attracted attention of medicinal chemists since 1960$^{th.}$ [Brown, 1965]. This interest has resulted in the emergence of a series of molecules with thiazolidinone core (Fig. 1.1), which are or were present on the pharmaceutical market: anti-diabetics such as Pioglitazone, Rosiglitazone, Lobegliazone [Lebovitz, 2019], medication for multiple sclerosis and psoriasis, e.g. Ponesimod [D'Ambrosio *et al.*, 2016], or treatment for diabetic neuropathy Epalrestat [Ramirez *et al.*, 2008]. The numerous reviews concerning the biological activity of 4-thiazolidinones corroborate the keen interest of medicinal chemists in this scaffold for the construction of drug-like molecules [Lesyk, 2020a], [Lesyk, 2020b], [Seboletswe *et al.*, 2023], [Nirwan *et al.*, 2019], [Manjal *et al.*, 2017].

a) ***b)***

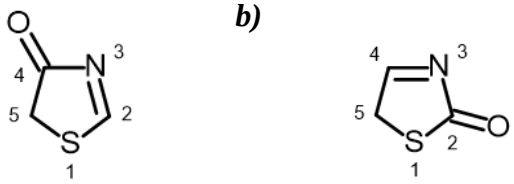


Fig. 1.1 1,3-Thiazol-4(5*H*)-one (a) and 1,3-thiazol-2(5*H*)-one (b) cores.

The 4-thiazolidinone nucleus diversified *via* an amino group as a linker (Fig. 1.2) is one of the most promising subsets of this group of heterocycles. As synthetically more accessible, 2-aminothiazolidinones are described in the literature incomparably more abundantly than the isomeric 4-aminothiazolidinones. The most important general methods to construct the 2-aminothiazolidinone system consist in the following steps (Fig. 1.3): (i) condensation of molecules bearing the N-C(=S)-N nucleophilic fragment, e.g. thiourea or thiosemicarbazide derivatives with equivalent dielectrophilic synthones; (ii) Dimroth-like rearrangement of 3-alkyl(aryl)pseudothiohydantoins, obtained after cyclization of N-alkyl(aryl)halogen-acetamides with thiocyanates; and (iii) nucleophilic substitution of the thiocarbonyl group of the rhodanine derivatives [Metwally *et al.*, 2010].

a) ***b)***

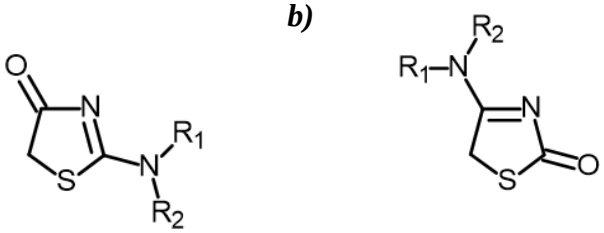


Fig. 1.2 (a) 2-Amino-1,3-thiazol-4(5*H*)-one and (b) 4-amino-1,3-thiazol-2(5*H*)-one cores.

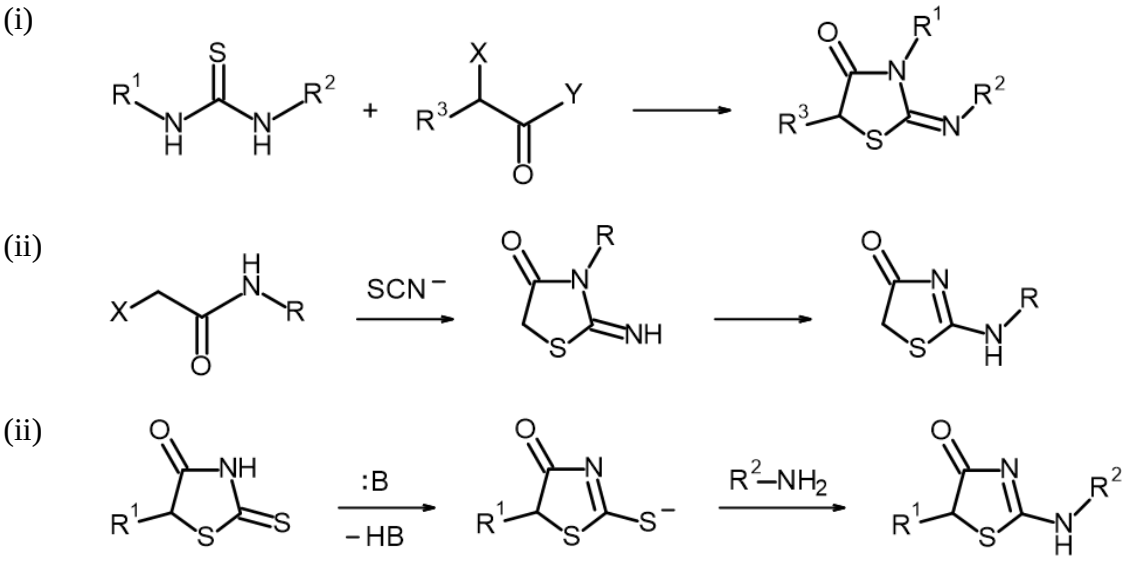


Fig. 1.3 Synthetic pathways for 2-aminothiazolidinones obtaining (X: Cl or Br, Y: usually alkoxy group).

Over the years, a large pool of diverse 2-aminothiazolidinones has been created and screened for a wide range of biological activities; the dominating activity profile turned out to have antiproliferative character. For instance, the pyridylaminopyridinylidene derivative (**I**)[1] was shown to selectively block the Aurora A kinase, a protein that participates in mitosis regulation and is a potential anticancer target [Kilchmann *et al.*, 2016]. The simple phenyl derivative with hydrazone bridge **II** was demonstrated to be an equally potent inhibitor of the Epidermal Derived Growth Factor as the reference drug Erlotinib [Lv *et al.*, 2010]. Rehulka and co-workers [Rehulka *et al.*, 2022] discovered the group of 5-halogenoarylidene-2-(4-hydroxyphenyl)aminothiazolidinones **III** as tubulin inhibitors, which induce the mitotic arrest inhibiting tubulin polymerization by attachment to the colchicine binding site. El-Naggar and co-workers showed that the isatino-4-thiazolidinone conjugate **IV** induces the expression of the pro-apoptotic protein Bax, while decreasing the level of the anti-apoptotic protein Bcl-2, with a strong cytotoxic effect against breast adenocarcinoma cell lines [El-Naggar *et al.*, 2018]. Another isatine–4-thiazolidinone hybrid (**V**) was demonstrated to be effective against colorectal and ovarian cancer, as well as renal carcinoma and melanoma cell lines [Fouad *et al.*, 2021]. The quinolone–4-thiazolidinone hybrid **VI** also exerts high activity against adenocarcinoma cell lines; docking simulations revealed that it binds a new chemotype of N-acetyl transferase (hNAT-1) protein [Kumar *et al.*, 2022]. Compound **VII** with indole scaffold attached *via* a methylidene linker to the 4-thiazolidinone C5 position was discovered to inhibit tubulin polimerisation and thus showing selective inhibition of colon cancer [Sigalapalli *et al.*, 2019]. Molecule **VIII**, also containing the indole backbone but attached at the C2 position *via* hydrazone bridge, showed high potential against MCF-7 cancer cell [de Oliveira *et al.*, 2017]. Molecule **IX**, comprised of 4-thiazolidinone, pyridine and piperazine cores, is active against prostate cancer cells [Demirci *et al.*, 2019]. A more branched pyridine derivative **X** has potential as an agent against glioblastoma cell lines [Campos *et al.*, 2023]. Pyrazolo-thiazolidinone

[1] *The formulas of compounds **I – XXIX** are shown in Fig. S1, Supplementary materials..*

hybrides were extensively studied as promising anticancer agents. Thus, a compound decorated with isatin fragment (**XI**) was shown to have a remarkable activity on human squamous carcinoma (SCC-15) cell line with concomitant caspase-3 activation [Szychowski *et al.*, 2017]. Pyrazoline–4-thiazolidinone hybrids with a pyrrole ring **XII** showed promising results against breast and colon cancer cell lines [Elewa *et al.*, 2022]. Chimeric pyrazole-based hybrid linked with piperazine moiety **XIII** was evaluated as promising selective VEGFR2 tyrosine kinase inhibitor, which is a pivotal protein for tumor vascularisation [El-Miligy *et al.*, 2017]. The purine-pyrazole containing molecule **XIV** shows excellent antiproliferative effect on several cancer cell lines [Afifi *et al*., 2019]. 4-Thiazolidinone-pyrazole hybrids connected *via* ylidene linkers (**XV)** demonstrated high potential against epithelial MDA-MB-231 cell line [Bhat *et al.*, 2018]. 2-Aminothiazolidinone with coumarine fragment at C5 position (**XVI**) promotes apoptosis in four cancer cell line [Sigalapalli *et al.*, 2021a], whereas the compound with the latter fragment at C2 position (**XVII**) exerted excellent activity on breast cancer cell lines MDA-MB-231 and BT-474, as well as lung cancer cell line A549 [Sigalapalli *et al.*, 2021b].

Worldwide spread of bacterial resistance to the available arsenal of antibiotics, has promoted intensive studies of 2-aminothiazolidinones as potential antimicrobials. Thus, the benzothiazole-containing compound **XVIII** is more active then the references ampicillin and streptomycin [Haroun *et al.*, 2018]; 2-thiazolyl derivatives **XIX** were discovered to possess remarkable activity against highly resistant strains of MRSA, *P. aeruginosa* and *E. coli* [Haroun *et al.*, 2021]. 2-Arylimino-3-aryl-thiazolidine-4-ones **XX** are explored as antbiofilm-formation agents, thought to afford an effective strategy for the eradication of biofilm-mediated infections that are resistant to conventional antibiotics. Incorporation of the 2-phenylfuran substituent greatly enhanced the antibiofilm activity of the thiazolidinone scaffold [Pan *et al.*, 2010]. Compounds **XXI** and **XXII**, bearing, respectively, allyl and naphtyl moieties at the 2-amino group, are able to inhibit bacterial aminoacyl-tRNA synthetases but not the mammalian analogues, which makes them candidates for first-in-class antimicrobials with new mode of action [Stana *et al.*, 2019].

2-Aminothiazolidinones with thiadiazole fragment were tested as potential antiviral agents. Compound **XXIII** exhibited strong inhibition of Hepatitis C virus in cell-based antiviral assays, and molecular docking studies showed that it can bind to the "thumb" pocket-II of the viral NS5B polymerase [Küçükgüzel *et al.*, 2013]. Conjugates of sydnones with 2-amino-thiazolidinones **XXIV** were found to have good antioxidant activity exerted *via* a radical scavenging mechanism [Shih *et al.*, 2004]. The perspective hexahydropyridazine derivative **XXV** was shown to have promising antiepileptic activity. Initially it was suggested to activate the K(+)-Cl(-) cotransporter KCC2, which reduces intracellular chloride concentration [Gagnon *et al.*, 2013], but later it was concluded to modulate the $GABA_A$ receptors [Cardarelli *et al.*, 2017]. The thiazole-bearing compound **XXVI** was evaluated to have prominent anticonvulsant activity [Mishchenko *et al.*, 2020].

Most of the above-mentioned papers devoted to the biological effects of 2-amino-thiazolidinones were published in the last ten years, showing a tremendous surge of interest and the growing potential of derivatives with this heterocyclic core, and also laying grounds for further interdisciplinary studies. Moreover, it is of note that a vast majority of the above-mentioned active compounds possess additional heterocyclic core (cores) and a double bond at position C5 of the heterocycle, which increases the biological activity and serves as a tool for

chemical diversification [Kaminskyy *et al.*, 2017], disproving the hypothesis about the law selectivity of such Michael acceptors.

4-Aminothiazolidinones

In contrast to 2-aminothiazolidinones, their structural isomers, 4-aminothiazolidinones are much less studied. It can be explained by the scarce synthetic tools for the construction of this scaffold. There is practically only one applicable method proposed by Komaritsa, namely nucleophilic substitution of the thiocarbonyl group of isorhodanines or thiorhodanines with an amino functional group [Komaritsa & Grishchuk, 1971]. The paucity of available synthetic methods is reflected in the law number of publications describing the biological activity of 4-aminothiazolidinones. The Lesyk group was the first to report the evaluation of the anticancer and antifibrotic activity of this group of compounds [Havrylyuk *et al.*, 2009], [Kaminskyy *et al.*, 2015], [Kaminskyy *et al.*, 2016]. They found the pyrazoline derivative **XXVII** to possess strong antitumor effect, especially on colon cancer cell lines. Pinson and co-workers found that the discussed derivatives were able to inhibit phosphoinositide 3-kinase PI3K [Pinson *et al.*, 2012]. In a patent application [Hasuoka & Ono, 2012], researchers have created a large library of 4-aminothiazolidinones with versatile heterocyclic cores at the C5 position in order to study their capacity to modulate the activity of estrogen-related receptors-α (ERR-α). Recently, it was discovered that 4-(3-chlorophenyl)-aminothiazolidinone derivative **XXVIII** is a selective inhibitor of *Mycobacterium tuberculosis* dihydroorotate dehydrogenase and, thus, a potent antitubercular agent [Alberti *et al.*, 2023]. 4-Phenylaminothiazolidinone with a furan-sulfonamide fragment **XXIX** was revealed to be a selective inhibitor of several isoforms of carbonic anhydrase [Angeli *et al.*, 2023]. These current findings have paved the way for further application of this poorly studied heterocyclic system in medicinal chemistry. My PhD dissertation is part of this effort.

Tautomerism of aminothiazolidinones

According to IUPAC, tautomerism (Gr., ταὐτός – same, μέρος - part) is ‘isomerism of the general form: where the isomers are readily interconvertible’ [Muller, 1994]. Katritzky and co-workers proposed to narrow this convention to interconversions with an activation energy below 20 kcal mol^{-1} [Katritzky *et al.*, 2010]. The term ‘tautomerism’ encompasses several types of double/single bonds migrations, such as prototropic, anionotropic, kationotropic, elementotropic, ring – chain tautomerism, or valence tautomerism [Elguero *et al.*, 2000], [Antonov, 2013], [Raczyńska *et al.*, 2005].

Prototropic (or proton) tautomerism, which refers to relocation of hydrogen atoms, is a common feature of both synthetic and natural compounds, and can be regarded as one of macroscopic manifestations of the quantum tunneling effect. It plays very significant role in almost all fields dealing with molecules: organic chemistry, biochemistry, molecular biology, pharmaceutical industry, etc. Thus, the importance of proton tautomerism can be exemplified by its impact on some vital processes in the living systems. The level of erroneous DNA base matches, like a shift towards the G*-T pair, where T is thymine and G* is a metastable enol tautomeric form of guanine; or C-A mismatches promoted by non-canonical tautomers of

cytosine and adenine in the active sites of DNA polymerases, can be highly elevated at certain conditions and lead to genetic mutations [Bebenek *et al.*, 2011], [Wang *et al.*, 2011].

This phenomenon is also important, albeit often underestimated, at each stage of the drug development process [Bharatam *et al.*, 2023]. Deposition in chemical databases of different tautomers as different substances confuses the field, complicates data managing and mining, and even leads to situations when the same chemical entity is sold as different products [Guasch *et al.*, 2016]. In the case of *in silico* calculations, the apparently slight changes of the chemical structure of different tautomers may lead to significantly different molecular properties, such as molecular geometry, pKa, hydrophilicity, or electron density distribution, hampering affinity estimations [Sayle, 2010]. It is worth noting that in some cases the tautomers of ligands established in molecular complexes are different from the tautomers of the free compounds [Brandstetter *et al.*, 2001], [Senthilkumar & Kolandaivel, 2002], [Yan *et al.*, 1998]. It has been estimated that about 25% of all drugs currently on the market may exist in two or more tautomeric forms [Martin, 2009] and according to Woods-Ryan *et al.*, 73% of the compounds in the drug subset of the Cambridge Structural Database (CSD) are susceptible to tautomerism [Woods-Ryan *et al.*, 2023]. Such active pharmaceutical ingredients (APIs) with long presence on the market, as omeprazole and ranitidine, are also objects of tautomeric studies because of the interest in the different bioavailabilities of the tautomeric forms of the APIs. The final industrial step, i.e., drug formulation, may also be influenced by tautomeric transitions, as pointedly illustrated by the formation of a new polymorphic phase upon grinding of the tautomerism-prone barbituric acid [Chierotti *et al.*, 2008].

Monosubstituted derivatives of aminothiazolidinones are also capable of existing in tautomeric equilibria (Fig. 1.4). The amino tautomer can be distinguished by the synperiplanar or antiperiplanar conformation around the single bond C4–N7, and the imino form can be found in the *E* or *Z* configuration of the C4–N7 double bond (Fig. 1.5). The experimental difficulties with adequate studies of tautomeric compounds and insufficient attention paid to this problem by chemists, all lead to discrepancies or even chaos in the literature and structural data bases, e.g., when the same compound from different sources was reported at the same conditions as different tautomers, confusing follow-up research, leading to dead-ends and waste of effort and resources. This problem concerns both 2-aminothiazolidinones [Mahbooby *et al.*, 2006], [Caille *et al.*, 2008], [Ranga *et al.*, 2013], [Arfeen *et al.*, 2016] and 4-aminothiazolidinones [Valls *et al.*, 1985], [Kaminskyy *et al.*, 2015]. Even structures deposited in the crystallographic databases (e.g., the CSD) require sometimes amendments to allocate the hydrogen atoms correctly, as illustrated by the case of 2-pyridylamino-thiazolidinones [Váňa *et al.*, 2009 and Gzella *et al.*, 2014] and ethyl 4-[(4-oxothiazolidin-2-yl)amino]benzoate [Behbeni & Ibrahim, 2012 and Gzella *et al.*, 2014].

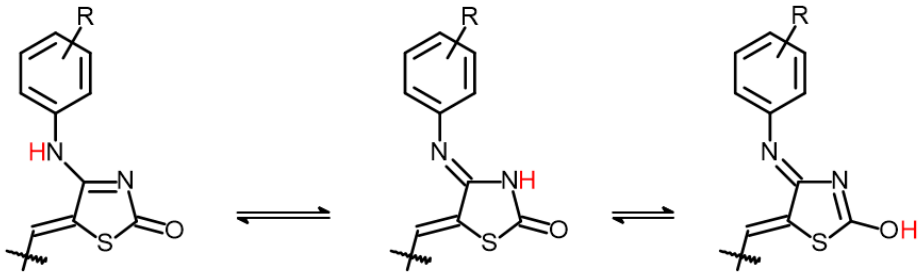


Fig. 1.4 Equilibrium of plausible tautomeric forms of 5-ylidine-4-phenylamino-thiazolidinones.

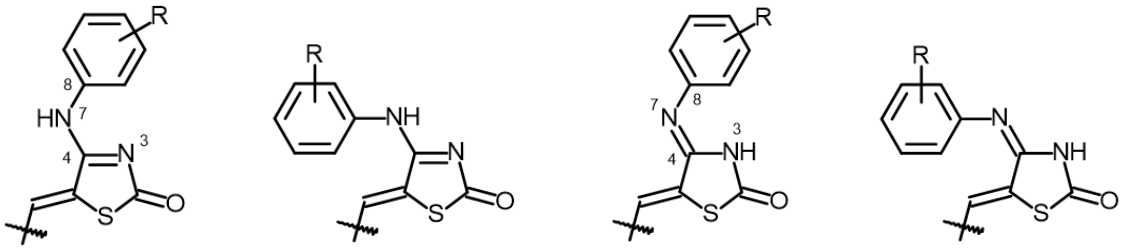


Fig. 1.5 Stereisomerism of 4-amino(imino)thiazolidinones.

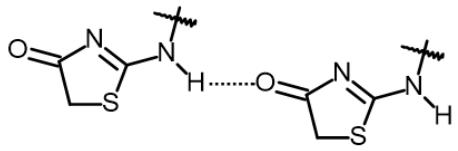


Fig. 1.6 N–H···O=C Intermolecular hydrogen bonds of 2-aminothiazolidinones in antyperiplanar conformation.

Fig. 1.7 N–H···N Hydrogen bonds between adjacent molecules of 2-aminothiazolidinonesin their crystals (A – refers to amino form, synperiplanar conformation, B – refers to imino form, *E*-configuration).

Due to their high pharmacological importance, the question of tautomerism of these heterocycles has been attacked since the 1970th. Ramsh, Engoyan and coworkers focused

mostly on simple 2-aminothiazolidinones. Applying IR, UV, and ^{1}H and ^{13}C NMR spectroscopy, they usually observed a mixture of tautomeric forms and a significant influence of the substituents' nature and position [Ramsh *et al.*, 1984], [Ramsh *et al.*, 1985], [Ramsh *et al.*, 1986], [Engoyan *et al.*, 1978]. The main conclusion was that in the liqiud state, 2-aminothiazolidinons exist as mixtures of the amino and imino tautomeric forms, and that electron-withdrawing substituents at the phenyl ring promote the imino tautomeric form [Minkin *et al.*, 2000]. More recently, X-ray crystallography was used to analyze the influence of the chemical character of the aromatic ring substituents in some 2-aminothiazolidinones. The results have partly corroborated the previous assertions about the imino form is promoted by electron withdrawing groups [Kowiel, 2015]. For 4-aminothiazolidinones some limited structural aspects of tautomerism have been mentioned only in the thesis of Pindela [Pindela, 2020], illustrating the yawning information gap between the isosteric 2- and 4-aminothiazolidinones. A similar comprehensive structural study of the effect of the substituents at position C5 on the tautomeric equilibrium of 4-aminothiazolidinones has not been presented so far.

A recent search of the Cambridge Structural Database (CSD, version 5.43, [Groom *et al.*, 2016]), returned 36 structures of 2-aminothiazolidinones and 12 structures of 2-iminothiazolidinones, including three structures with incorrectly specified tautomeric form (refcodes: GACXOZ, HEGLUC, IMTAZO). Allocation of hydrogen atoms, which are crucial for the tautomeric forms, using single-crystal X-ray diffraction (which maps the electron density in the molecules) is difficult, although not impossible when very high experimental accuracy is sought, because of the presence of just one formal electron. However, the position of H atoms may be inferred (i) from the location of the X-H bonding orbitals, (ii) from the molecular geometry that is dictated by the location of the mobile H atoms, and (iii) from the patterns of donor/acceptor hydrogen bonds. Hence, analysis of hydrogen bonding geometry in crystals is a reliable tool for accurate location of these protons. A preliminary analysis of CSD-deposited structures of 2-amino(imino)-thiazolidinones revealed that N–H···O=C< hydrogen bonds are present in compounds with antiperiplanar conformation (existing only as amino tautomers, Fig. 1.6). At the same time, molecules with synperiplanar conformation (which can be adapted by both the amino or imino tautomeric forms, and termed *E*-configuration), are connected by hydrogen bonds between the N–H···N amidine fragments, which is not informative because of the resonance effect and proton oscillation within this dimeric system (Fig. 1.7). It is worth noting that deformation of the C2–N3 and C2–N6 bonds, which is affected by the electron resonance, was observed solely for compounds in the amino form, regardless of the assumed conformation. For 56 analyzed structures with $R \leq 0.075$, the C2–N3 and C2–N6 bond lengths were on average 1.316(1) and 1.323(1) Å, respectively (Table S3), i.e., intermediate between the reference values for a single [1.374(1) Å] and double [1.279(1) Å] Csp^2–N bond lengths (Table S4). Interestingly, for compounds in the imino tautomeric form, this hybridization of single and double bounds was not observed, as no structure with the *Z*-configuration were found.

For 4-aminothiazolidinones, there are just 6 CSD-deposited structures, all of them in the amino tautomeric form, highlighting the paucity of the corresponding synthetic and structural data (Table S5).

2. AIMS OF THE WORK

The primary objective of my doctoral project has been to study the amine-imine proton tautomerism and stereoisomerism of carefully selected set of 5-ylidene-4-phenylamino-1,3-thiazol-2(5*H*)-one derivatives (Fig. 3.1).

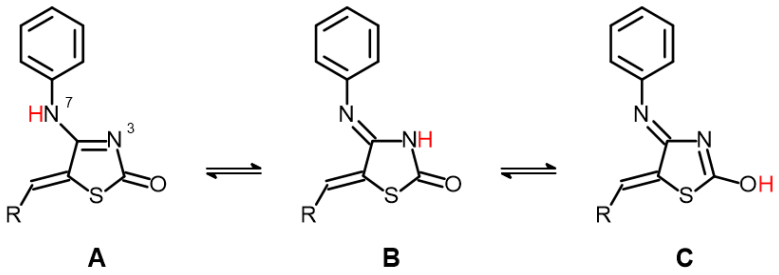


Fig. 2.1 Possible tautomeric structures of 5-ylidene-1,3-thiazol-2(5*H*)-ones.

Achievement of the main research objective leads through the following partial tasks:

- ✓ Optimization of the synthesis protocols and preparation of the compounds selected for further analysis.
- ✓ Isolation and purification of the finished products.
- ✓ Submission of the new compounds for FT-IR and ^{1}H and ^{13}C NMR experiments.
- ✓ Selection of solvents and optimization of crystallization conditions, for the production of well-diffracting single crystals; concomitant evaluation of potential polymorphic forms of the compounds under study.
- ✓ X-Ray diffraction experiments, carried out at low temperature (130 K).
- ✓ Solution of the phase problem and least-squares refinement of the crystal structures.
- ✓ Analysis and deposition of the crystal structures in the Cambridge Structural Database (CSD).
- ✓ Inclusion of the results of quantum chemical calculations in the analyses.
- ✓ Comparative studies of conformational isomerism, *E*/*Z* isomerism, and mesomerismin the newly determined molecular structures.
- ✓ Determination of the tautomeric preferences in both the liquid and solid phases.
- ✓ Detailed study of supramolecular self-organization of the new molecules in their crystals, to assess the modes of aggregation and the role of directional interactions, such as hydrogen bonds, in the aggregation patterns.

In order to form a comprehensive view of the phenomena of proton tautomerism, stereoisomerism, mesomerism, and possible polymorphism in a given class of compounds, it is important to properly select the molecular objects to be studied as well as the experimental methods to be used. Only a sufficiently diverse variety of compounds and experimental techniques will guarantee a meaningful analysis of the problems. Research techniques such X-ray crystallography, ^{1}H and ^{13}C NMR and FT-IR spectroscopy, as well as quantum-chemical calculations, were utilized in this project to describe the above molecular phenomenon in a thorough and systematic way.

For the analysis, first, derivatives of 4-phenylamino-1,3-thiazol-2(5*H*)-one with an unsubstituted phenyl system and an extra ylidene group at the C5 position with electron-

withdrawing methoxycarbonylmethylidene substituent (**2**) and an electron-donor dimethylaminomethylidene substituent (**1**) were selected. Other compounds synthesized for the analysis included 5-dimethylaminomethylidene-4-phenylamino-1,3-thiazol-2(5*H*)-one derivatives with electron-donor substituents (-OH: **3**–**5**; -OCH_3: **6**–**8,** see section Main results and discussion) occupying successively the *ortho*, *meta* and *para* positions, and with electron-acceptor substituent (-CF_3: **9**, **10**) occupying the *meta* and *para* positions in the phenyl ring.

The author of the dissertation is convinced that the results will significantly advance our understanding of the phenomenon of proton tautomerism and stereoisomerism in the class of five-membered heterocyclic compounds with nitrogen and sulphur heteroatoms, particularly in the group of the selected 4-phenylamino-1,3-thiazol-2(5*H*)-one derivatives. Additionally, the author anticipates that the results will aid in the design and synthesis of new heterocyclic compounds with the thiazolone/thiazolidinone system exhibiting pharmacological activity.

3. METHODOLOGICAL APPROACHES APPLIED FOR RESEARCH

Synthesis

To afford the title 5-ylidene derivatives of 4-phenylamino-1,3-thiazol-2(5*H*)-ones it has been applied the nucleophylic substitution of isorhodanine with aromatic amines. This is the practically sole approach for such 4-amino derivatives of thiazolidinones, for the first time proposed by Komaritsa in 1971 starting from isorhodanine, which was also discovered by him earlier [Komaritsa & Grishchuk, 1971] as a product of thionation of 2,4-thiazolidinedione with P_2S_5. The modification of C5 position of the heterocyclic core with substituents possessing opposite electronic effects (dimethylaminomethylidene or methoxycarbonylmethylidne fragment) was carried out *via* Knoevenagel-type reaction with dimethylformamide dimethyl acetal (DMF-DMA) or glyoxylic acid with subsequent esterification respectively. The synthetic conditions were optimized for the best yields of the target compounds. Purification of the products on the each synthetic step was performed by normal phase column chromatography ($CHCl_3$-MeOH) or by recrystallization from proper solvent. Melting points were measured on Boetius apparatus and were uncorrected.

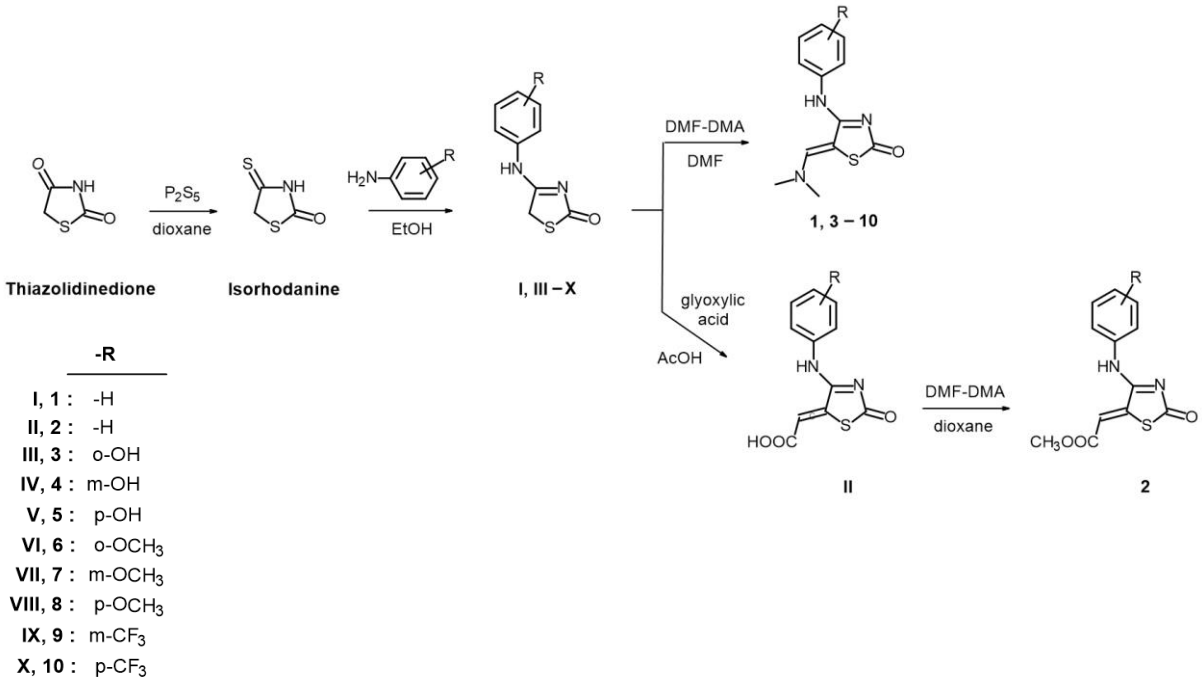


Figure 3.1 Synthesis of 4-(*R*-phenyl)amino-1,3-thiazol-2(5*H*)-one derivatives (**1** – **10**)

Crystallization

Crystals suitable for single crystal X-ray diffractometry were obtained by slow evaporation of the solvent. As the title compounds posses very low water solubility, the most accesable solvents for crystal growth were dimethylaformamide (DMF) and, to a lesser extent, alkohols (MeOH or EtOH) and dimethyl sulfoxide (DMSO). For 5-dimethylaminomethylidene-4-phenylamino-1,3-thiazol-5(*H*)-one (publication D-2) it was also found to from two

polymorph from DMF solution, but not from dioxane, which was confirmed by powder X-ray diffraction (PXRD) experiment.

X-ray analysis

The single crystal X-ray measurements were perfomed on SuperNova Rigaku diffractometer equipped with Dual Atlas detector (all compounds, described in articles except of compound **3** in publication D-2) and Rigaku XtaLAB Synergy-R diffractometer equipped with a Rigaku HyPix-6000HE detector (for compound 3 in publication D-2). The crystal structures of the title compounds were solved by the dual-space direct methods algorithm (SHELXT [Sheldrick, 2015a], [Dolomanov *et al.*, 2009], [Farrugia, 2012]) and refined against F^2 using all data (SHELXL [Sheldrick, 2015b], [Dolomanov *et al.*, 2009], [Farrugia, 2012]). The positions of the H atoms bonded to the N and O ones were obtained from difference Fourier maps and were refined freely with isotropic displacement parameters. The remaining H atoms were placed geometrically at calculated positions and refined using the riding model, with the following parametrization: C—H 0.98 Å (CH_3), 0.95 Å (Csp^2H) and $U_{iso}(H) = 1.2U_{eq}(C)$ or $1.5U_{eq}(C)$ for methyl protons. The methyl groups were refined as rotatable rigid groups. Molecular illustrations were prepared using ORTEP-3 for Windows [Farrugia, 2012]. The material for publication was prepared using WINGX [Farrugia, 2012] and PLATON [Spek, 2009]. The structures of the title compounds were deposited at the CCDC with numbers CCDC2160230 (**1-I)**, CCDC2160229 (**1-II)**, CCDC2160228 (**2**), CCDC2233456 (**3**), CCDC2233457 (**4**), CCDC2233458 (**5**), CCDC2252019 (**6**), CCDC2252020 (**7**), CCDC2252021 (**8**), CCDC2293072 (**9**), CCDC2293071 (**10**), CCDC1995144 (**11**).

The PXRD patterns of the samples were recorded on a Bruker AXS D2 Phaser diffractometer with CuK_α radiation (λ = 1.54060 Å) and 1 mm slit. The operating voltage and current were 30 kV and 10 mA, respectively. The samples were scanned from 2θ = 5 to 45°, with 0.02° step size, 2 s/step counting rate, and sample spinning.

Spectroscopic studies

Nuclear Magnetic Resonance (NMR) spectra were recorded on Bruker Avance (^{1}H at 600 and ^{13}C at 150 MHz) and Bruker Avance 3 (^{1}H at 700 and ^{13}C at 175 MHz) instruments, all spectra has been carried out in DMSO-d_6 solutions. Chemical shifts (δ) were given in ppm units relative to tetramethylsilane as reference (0.00).

FT-IR spectra has been performed on a Bruker Equinox 55 spectrometer (publications D-2, D-3 and D-4) or on a Nicolet iN10FX spectrometer (publication D-1) in the spectral range from 7000 to 400 cm^{-1} (publications D-2, D-3 and D-4), in the range of 7500 to 400 cm^{-1} (compounds in publication D-5), with a resolution of 2 cm^{-1} in the matrix of KBr. To interpret the bands observed in the experimental spectra, normal modes calculations were employed using the DFT [ωB97X-D/6-311G(d,p)] method. Two ranges were analyzed, namely, 3700–2300 cm^{-1} and 1750–1320 cm^{-1}, the most important for identification of tautomeric form bands are present.

Computational methods

The CrystalExplorer21 [Spackman *et al.*, 2021] program was applied to explore intermolecular interactions and crystal packing. The Hirshfeld area is defined as the area around a molecule in which the weight function w(r)≥0.5 [Hirshfeld, 1977]. Quantum mechanical calculations in vacuum and in dimethylsulfoxide (DMSO) were carried out in the Gaussian09 package [Gaussian, 2016] applying crystallographic models for geometry optimization. A hybrid function with long-range ωB97XD correction [Chai & Head-Gordon, 2008a, Chai & Head-Gordon, 2008b] and the Pople 6–311G(d,p) basis set [Ditchfield *et al.*, 1971] were used to establish and optimize the normal modes. Potential energy scans (PES) around selected bonds were used for conformational analysis. AIMALL software [Todd & Keith, 2017] was used to analyze hydrogen bonds using the Quantum Theory of Atoms in Molecules (QTAiM) [Bader, 1994]. Parameters such as electron density (ρBCP), the Laplacian of the electron density ($\Delta\rho$BCP), potential electron energy density (*V*BCP), kinetic electron energy density (*G*BCP), and the total electron energy density (*H*BCP) at the critical points were analyzed for the X-ray geometry of the title molecules.

Biological methods

Anticancer assays were carried out on a panel of approximately sixty human tumor cell lines referring to nine neoplastic diseases, by treating the cells with 10^{-5} M solutions of each compound and incubation the cell cultures for 48 h. At the final point, cell cultures were stained with the sulforhodamine B (SRB) protein-binding dye and cell growth/viability was evaluated spectrophotometrically versus untreated controls. Using absorbance measurements [time zero (Tz), control growth in the absence of drug (C), and test growth in the presence of drug (Ti)], the percent growth was calculated for each drug concentration. Percent growth inhibition was calculated as: [(Ti - Tz) / (C - Tz)] x 100 for concentrations for which Ti ≥ Tz, [(Ti - Tz) / Tz] x 100 for concentrations for which Ti < Tz [Monks *et al.*, 1991], [Boyd *et al.*, 1995], [Shoemaker *et al.*, 2006].

4. MAIN RESULTS AND DISCUSSION

The doctoral thesis consists of five research articles D-1 to D-5 [Pyrih *et al.*, 2021, 2023a, 2023b, 2023c, 2024], three of them published in the scientific journal *Journal of Molecular Structure*, and two in the IUCr journals *Acta Crystallographica Section B: Structural Science, Crystal Engineering and Materials* and *Acta Crystallographica Section C: Structural Chemistry*. This set of papers is preceded by paper D-0 [Kaminskyy *et al.*, 2017], published in *SynLett* before the formal commencement of the Doctoral Studies, which should be considered as an introduction to the whole series. Papers D1 to D-5 present the synthesis and comprehensive experimental analysis, using spectroscopic (^{1}H and ^{13}C NMR; FT-IR) and crystallographic (single-crystal and powder X-ray diffraction) methods, of as many as 20 derivatives of 5-ylidene-4-phenylamino-1,3-thiazol-2(5*H*)-one. The main goal of the project was the elucidation of proton tautomerism and stereoisomerism of differently substituted 4-phenylamino-1,3-thiazol-2(5*H*)-one derivatives.

In order to gain a comprehensive insight into the tautomeric effect of the title compounds, 5-ylidene-4-phenylamino-1,3-thiazol-2(5*H*)-one derivatives diversified at the C5 position of the thiazolidinone core with substituents of opposite properties: electron donating (dimethylaminomethylidene) and electron withdrawing (methoxycarbonylmethylidene), were created and analyzed [Pyrih *et al.*, 2023a]. Also, a series of derivatives of 5-dimethyl-aminomethylidene-4-phenylamino-1,3-thiazol-2(5*H*)-one decorated with the -OH [Pyrih *et al.*, 2023b] and -OCH_3 [Pyrih *et al.*, 2024] groups at the *ortho*, *meta* and *para* positions of the phenyl ring, as well as hybrid molecules with diaryl(hetaryl)pyrazoline fragment linked by a methylidene bridge to the C5 position of the thiazlolidinone system were synthesized. As a general conclusion, structure – tautomeric form relationships have been deduced. For some of the new compounds their biological potential was evaluated.

4.1. One-Pot Synthesis of 5-Ene-4-aminothiazol-2(5*H*)-ones and Chromeno[2,3-*d*]-thiazol-2-ones (publication D-0)

My work, dedicated to the synthesis and structural analysis of 4-thiazolidinones, especially of derivatives of 4-amino-1,3-thiazol-2(5*H*)-one, started before the formal commencement of my PhD project at the Faculty of Chemistry, Adam Mickiewicz University in Poznan. The early research was conducted as part of scientific collaboration between Poznan Medical University and Danylo Halytskyi Lviv Medical University, and resulted in the synthesis and X-ray structural analysis of 4-thioxo-1,3-thiazolidin-2-one (isorhodanine) (**1**), 4-amino-1,3-thiazol-2(5*H*)-one (**2**), and 5-(4-chlorobenzylidene)-4-(2-hydroxyethyl)-amino-1,3-thiazol-2(5*H*)-one (**3a**) in collaboration with prof. Andrzej Gzella and Aneta Suseł (Pindela). The above new compounds were studied by single-crystal X-ray diffractometry (**1**, **2**, **3a**), FT-IR (**3a**, solid phase) spectroscopy, as well as ^{1}H and ^{13}C NMR (**2**, **3a**–**f**, DMSO solution) and ^{1}H and ^{15}N NMR (**2**, DMSO solution) spectroscopy. The results were published in the research article „One-Pot Synthesis of 5-Ene-4-aminothiazol-2(5*H*)-ones and Chromeno[2,3-*d*]thiazol-2-one", published in *SynLett* [Kaminskyy *et al.*, 2017], with my co-authorship. The compounds **1** and **2** mentioned above have been used as substrates for the subsequent synthesis of

N-substituted 4-amino-1,3-thiazol-2(5*H*)-ones [Komaritsa & Grishchuk, 1971]. Compound **3a** together with the remaining five preparations described in D-0, are potential biologically active compounds obtained by a modified one-pot reaction. This three-component approach allows one to avoid the isolation of the intermediate (Fig. 4.1.1) and S-methylation of the 5-ene-4-thioxo-2-thiazolidinones.

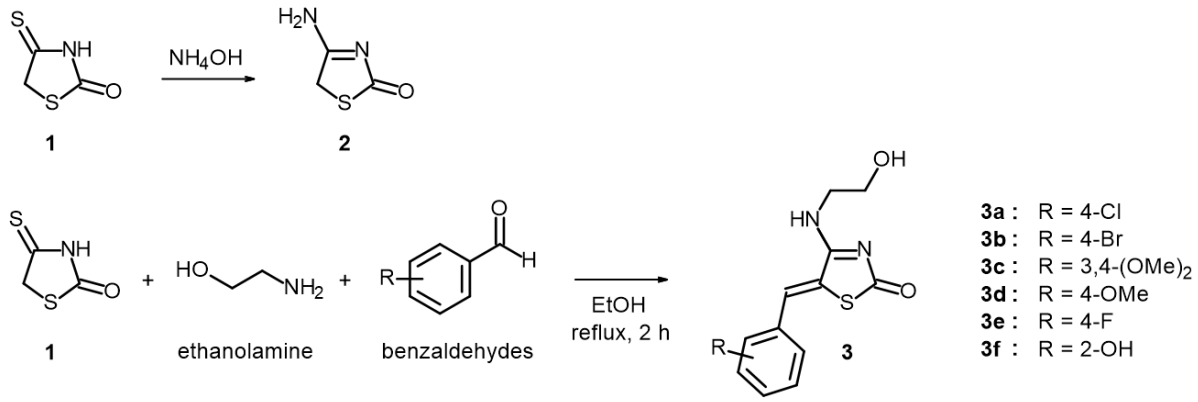


Fig. 4.1.1 Three-component synthesis of 5-ylidene-4-(2-hydroxyethylamino)-thiazol-2(5*H*)-ones (**3a**–**f**).

It is worth noting that all the studied compounds **1, 2, 3a**–**f** are susceptible to proton tautomerism whereby compounds **1** and **2** can exist in five tautomeric forms (Fig. 4.1.2), and compounds **3a–f** in three tautomeric forms (Fig. 4.1.3).

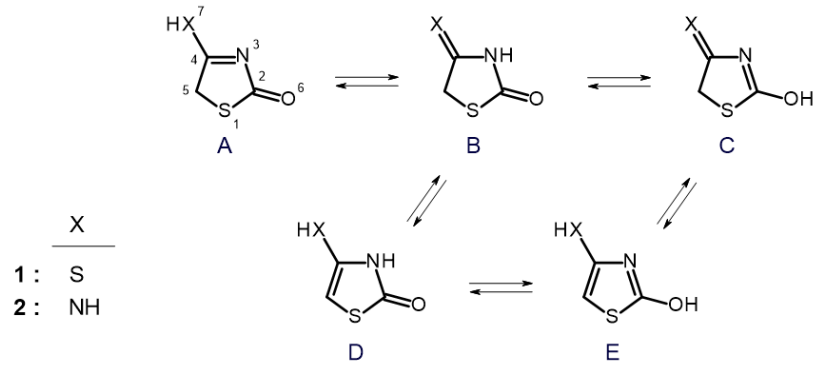


Fig. 4.1.2 Possible tautomeric structures of isorhodanine (4-sulfanylidene-1,3-thiazolidin-2-one) (**1**) and 4-amino-1,2-thiazol-2(5*H*)-one (**2**).

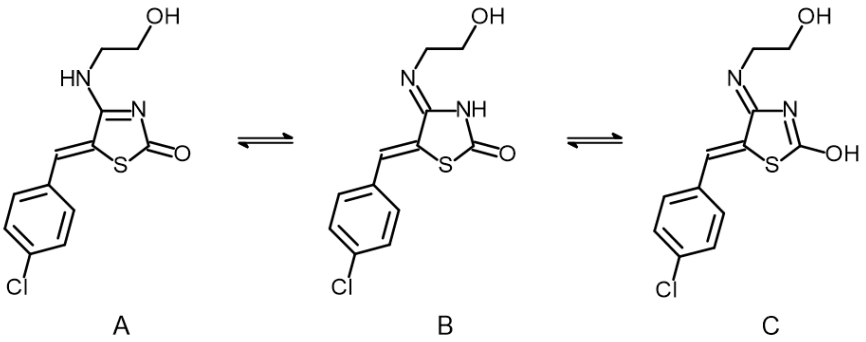


Fig. 4.1.3 Possible tautomeric structures of 5-(4-chlorobenzylidene)-4-(2-hydroxyethyl)-amino-1,3-thiazol-2(5*H*)-one (**3a**).

The X-ray analysis and solid state FT-IR spectroscopy, both show that isorhodanine (**1**) exists in the solid phase in the form with a secondary amide group, while 4-amino-1,3-thiazol-2(5*H*)-one (**2**) and 5-(4-chlorobenzylidene)-4-(2-hydroxyethyl)amino-1,3-thiazol-2(5*H*)-one (**3a**) exist as amine tautomer, with an exocyclic amine N7 atom and an endocyclic N3 atom of imine character. Based on ^{1}H and ^{13}C NMR spectra, it was found that compounds **1**, **2**, **3a–f** also exist in the amine tautomeric form in DMSO solutions. To address the proton tautomerism problem, investigations of compound **2** utilizing ^{1}H and ^{13}C NMR techniques were insufficient. Consequently, I also took advantage of the ^{1}H,^{15}N HSQC NMR correlation spectrum, which allowed me to reach the ultimate conclusion that the molecule under study exists in DMSO solution in the amine tautomeric form with two H atoms at the exocyclic N7 atom. This results contradicted earlier findings that 4-amino-1,3-thiazol-2-one exists in liquid phase in the imine tautomeric form (Valls et al., 1985).

Another interesting finding relates to crystals used in diffractometric research. Thus, single crystals suitable for X-ray analysis were obtained, as in all following cases, by slow evaporation of the solutions in ethanol (polymorph **1-I**), DMF (polymorph **1-II**), water (**2**), or dioxane (**3**). The crystallographic analysis established that the crystals of **1-I** belong to the monoclinic system and the centrosymmetric space group $P2_1/n$, whereas crystals of **1-II** belong to the orthorhombic system and the non-centrosymmetric space group $Pna2_1$. Interestingly, the shape and the size of the unit cells of polymorphs **1-I** and **1-II** are almost identical, in spite of the different symmetry (Table 4.1.1).

Table 4.1.1 Selected crystallographic data for polymorphs **1-I** and **1-II**.

	1-I	**1-II**
Chemical formula	$C_3H_3NOS_2$	$C_3H_3NOS_2$
M_r	133,18	133,18
Crystal system, space group	Monoclinic, $P2_1/c$	Orthorhombic, $Pna2_1$
Temperature(K)	293(2)	130.0(1)
a, b, c (Å)	4.1194(5), 21.874(2), 5.7164(5)	21.5791(14), 4.1545(2), 5.6447(3)
α, β, γ (°)	90, 91.969(10), 90	90, 90, 90
V (Å^3)	514.79(9)	506.05(5)
Z, Z'	4, 1	4, 1

Based on the X-ray analysis, it was found that the isorhodanine molecules in the crystals of both polymorphs (**1-I** and **1-II**) are connected by N3–H3···O6^{i} hydrogen bonds to form ribbons (Fig. 4.1.4). Despite the different symmetry of crystals **1-I** and **1-II**, their two-dimensional similarity was found by the approach of (Fábián and Kálmán, 2004).

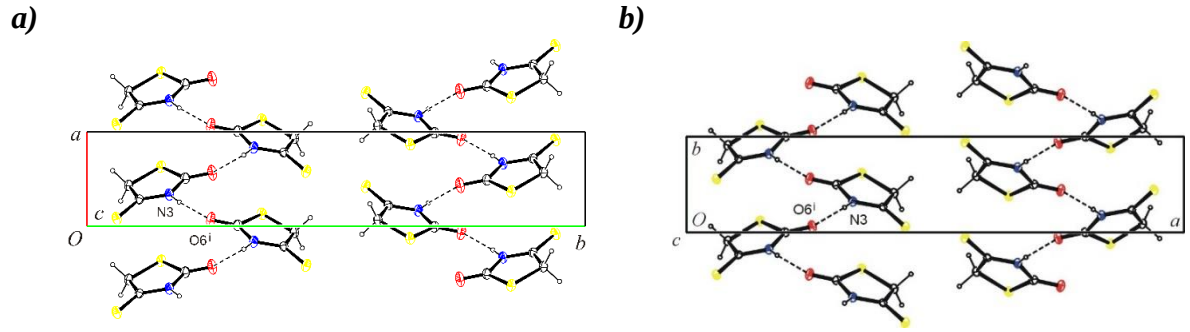


Fig. 4.1.4 ORTEP views along *c* axes of the unit cells of polymorphs **1-I** (a) and **1-II** (b), showing ribbons of hydrogen-bonded molecules. Symmetry codes: (i) –1/2+*x*,1/2–*y*,–1/2+*z* (**1-I**), 1/2–*x*,–1/2+*y*,1/2+*z* (**1-II**).

For crystal structure of **3a**, the asymmetric unit was found to contain independent molecules A and B, in which the phenyl ring of the (4-chlorophenyl)methylidene residue displays disorder (Fig. 4.1.5). The two alternative conformations are related by rotation of the phenyl ring around the C12–C15 bond, whereby molecule A with an alternative phenyl orientation essentially becomes molecule B and, conversely, molecule B with an alternative phenyl arrangement becomes molecule A. This means that there are unit cells in the crystal (s.o.f. = 0.05) in which the independent molecules A and B swap places.

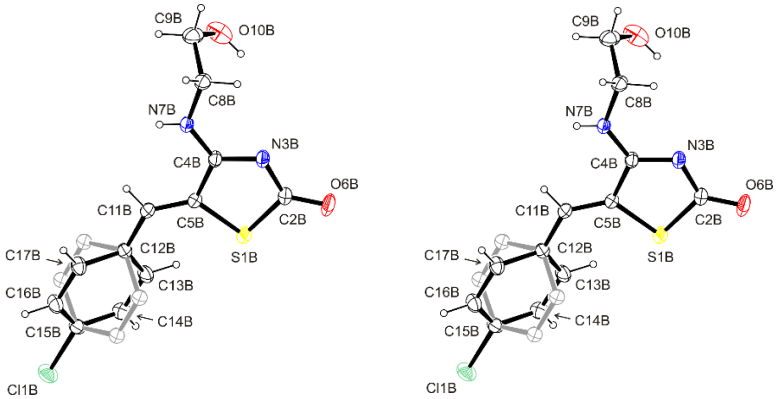


Fig. 4.1.5 ORTEP view of the independent molecules of **3a** (left - A, right - B) with atom labelling scheme[1]. The alternative orientations of the phenyl ring are in gray.

[1]*The phenyl rings in the alternative positions in independent molecules A and B consist of C12A,C13C,C14C,C15A,C16C,C17C and C12B,C13D,C14D,C15B,C16D,C17D atoms, respectively.*

4.2 Proton tautomerism and stereoisomerism of 4-amino-1,3-thiazol-2(5*H*)-one derivatives bearing substituents with opposite electronic effects: Synthesis, structure and spectroscopic studies (publication D-1)

Publication D-1 reports the synthesis as well as structural and spectroscopic analysis of two ylidene derivatives of 4-amino-2-thiazolone. Single-crystal and powder X-ray analysis, FT-IR, ^{1}H and ^{13}C NMR spectroscopic methods, as well as theoretical DFT calculations were applied in order to evaluate the influence of the substituent at the C5 position of the thiazolidinone core on the electronic structure and prototropic tautomerism.

To obtain the title 5-ylidene derivatives of 4-phenylamino-1,3-thiazol-2(5*H*)-one, nucleophilic substitution of isorhodanine with aniline was applied. This is practically the sole approach for such 4-amino derivatives of thiazolidinones, pioneered by Komaritsa, who also discovered isorhodanine [Komaritsa & Grishchuk, 1971] as a product of thionation of 2,4-thiazolidinedione with P_2S_5. The modification of the C5 position with substituents possessing opposite electronic effects (dimethylaminomethylidene or methoxycarbonylmethylidne) was carried out *via* Knoevenagel-type reaction with dimethylformamide dimethyl acetal (DMF-DMA) or glyoxylic acid with subsequent esterification. The synthetic conditions were optimized for the best yields of the target compounds (Fig. 3.1, section 3: Methodological approaches applied for research)

The X-ray diffraction analysis of compounds **1** and **2** was carried out on crystals obtained by slow evaporation of the solutions in DMF (**1**) or DMSO (**2**). It was established that compound **1** crystallizes as two polymorphs **1-I** and **1-II**, which belong to monoclinic non-centrosymmetric *Cc* (**1-I**) and centrosymmetric $P2_1/n$ (**1-II**) space groups, compound **2** crystallizes in the monoclinic centrosymmetric $P2_1/c$ space group. The molecules in the crystals of both polymorphs have the same tautomeric shape and identical conformation, despite the lower quality of the structure identified for polymorph **1-II** (R = 0.0867). Thus, the higher-precision structure **1-I** served as the basis for their full description.

The structural and spectroscopic studies confirmed the synthesis of the title compounds **1** and **2**, and the existence of molecules of both compounds in the amino tautomeric form, both in the solid and liquid (DMSO) phases (structure A, Fig. 4.2.1).

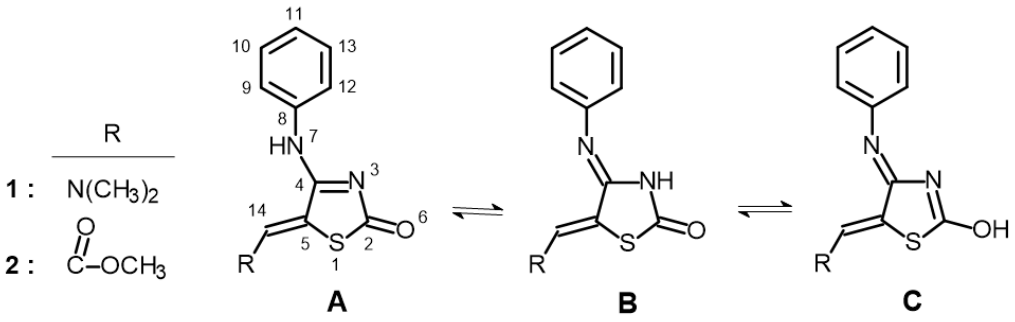
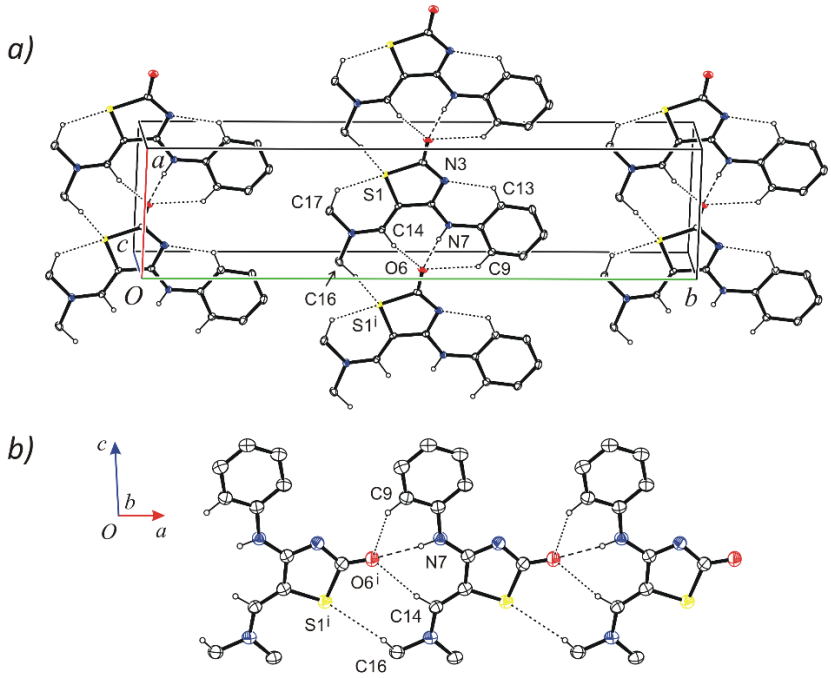


Fig. 4.2.1 Possible tautomeric structures of **1** and **2**.

In the X-ray studies the positions of the amidine H atoms in the crystals of **1** and **2** were obtained from difference Fourier maps and were refined freely. Their locations were confirmed *via* N7–H7···O6^{i} hydrogen bonds (Fig. 4.2.2), in which the proton acceptor is the carbonyl

oxygen atom O6. The presence of the molecules in amine tautomeric form is also indicated by the involvement of the imine N3 atom in the formation of the intramolecular C13–H13···N3 hydrogen bond, in which the N atom acts as a proton acceptor and the CH group in the *ortho* position of the phenyl ring as a proton donor (Fig. 4.2.3). This hydrogen bond can form because of the synperiplanar conformation of the phenylamine residue and the very slight tilting of the phenyl ring away from the mean plane of the thiazolone system (dihedral angle 2.32(5)º in **1-I**, 13.68(3)º in **2**). It is worth noting at this point that the theoretical conformational analysis of isolated molecules of **1** and **2** carried out using the DFT(ωB97X-D/6-311G(d,p)) method shows that the potential energy scan (PES) for a rotation around the N7–C8 single bond has minima for the C4–N7–C8–C9 torsional angle of 177.9° and -2.0° for **1**, and 177.9° and -2.9° for **2**. The found spatial structure of the molecules of **1** and **2** (the almost flat molecular shape and synperiplanar conformation of the phenylamine residue) prevents the appearance of the imine form in the studied crystals. According to the noted molecular geometry of **1** and **2**, the distance (C9/13-)H···H(-N3) [C-H in the *ortho* position of the phenyl system and H-N of the thiazolone system] would be only 1.5–1.6 Å, indicating a spatial lock, if the H atom was to appear in the N3 position (at the endocyclic N atom).



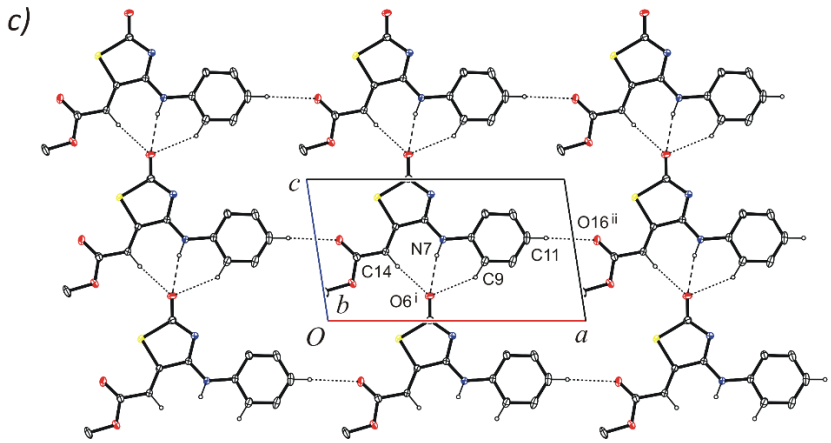


Fig. 4.2.2 Hydrogen bonds N7–H7···O6^{i} in **1-I** (a), **1-II** (b) and **2** (c). Symmetry codes (i) for **1-I, 1-II**: –1+*x*,*y*,*z*, for **2**: *x*,*y*,1+*z*.

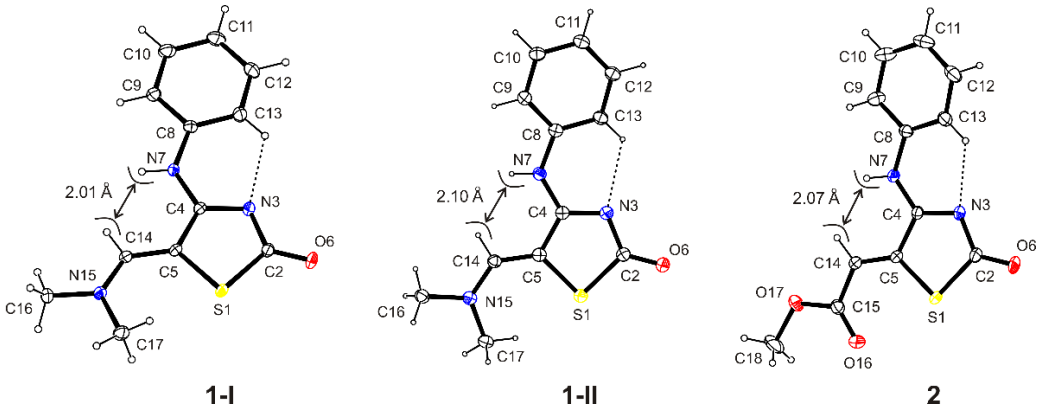


Fig. 4.2.3 ORTEP views of the molecules of **1-I**, **1-II**, and **2**, showing the atomic labeling schemes. Non-H atoms are drawn as 30% probability displacement ellipsoids, and H atoms are drawn as spheres of arbitrary size.

Observations relating to the amine tautomeric form in the crystals of **1** and **2** made by means of the X-ray method were confirmed by the studies based on solid-state FT-IR analysis supported by theoretical calculations.

The fact that the molecules of **1** and **2** exist in the solid-state in the same amine tautomeric form as 4-amino-1,3-thiazol-2(5*H*)-one [Kaminskyy *et al.*, 2017] and 4-phenylamino-1,3-thiazol-2(5*H*)-one [Pindela, 2020] means that neither the displacement of the amine group (-NH_2) for a phenylamine group (-NH-Ph), nor an additional presence at C5 of either electron-withdrawing or electron-donating substituents, does not alter the tautomeric form from amino to imino one.

According to the X-ray investigations, the ylidene substituents in the C5 position of the thiazolidinone core have an impact on the electronic structure of the molecule by taking part in the resonance effect with the carbonylimine system. Wherein only electron-donating dimethylaminomethylidene (**1-I**) substituent was shown to contribute to the resonance effect (Fig. 4.2.4). Unlike the dimethylaminomethylidene substituent, the methoxycarbonyl-methylidene electron-withdrawing substituent in the molecule of **2** is unlikely to participate in

the resonance with the carbonylimino group. Therefore, in compound **2**, the resonance effect mediated by the carbonylimino group this time is mainly directed to the exocyclic amine nitrogen atom N7 (Fig. 4.2.4).

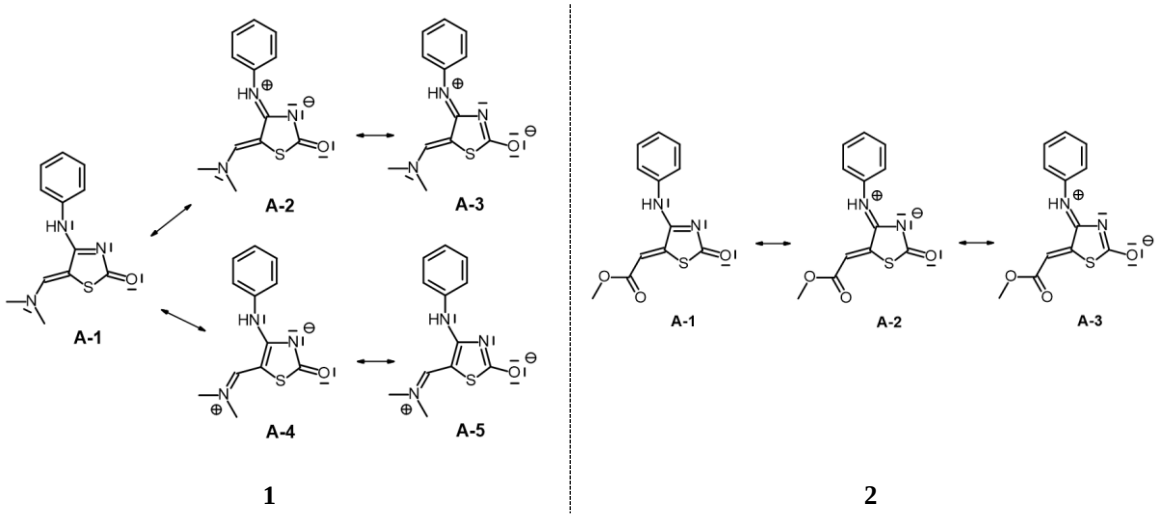


Fig. 4.2.4 Electron resonance structures of compounds **1** and **2**.

Interestingly, it was found that the participation of the ylidene substituent in the resonance effect with the carbonylimine group or its absence affect the chemical shift of the amidine H atom in the ^{1}H NMR spectrum. Thus, for compound **1** with an electron-donor dimethylaminomethylidene substituent, the amidine H atom was found in the ^{1}H NMR spectrum at δ = 9.50 ppm, while for compound **2** with an electron-acceptor methoxycarbonylmethylidene substituent and the H volume was found at δ = 10.93 ppm.

4.3 Proton tautomerism in 5-dimethylaminomethylidene-4-(*o*-,*m*-,*p*-hydroxyphenyl)-amino-1,3-thiazol-2(5*H*)-ones: synthesis, crystal structure and spectroscopic studies (publication D-2)

The publication D-2 describes the synthesis and structural and spectroscopic analysis of 4-(*o*-,*m*-,*p*-hydroxyphenyl)amino-1,3-thiazol-2(5*H*)-one derivatives containing an additional dimethylaminomethylidene substituent at the C5 position of the thiazolone system.

The X-ray crystallographic, FT-IR, ^{1}H and ^{13}C NMR spectroscopic analyses, and DFT theoretical calculations were used to evaluate the impact of the OH group in the phenyl ring at the *ortho*, *meta*, and *para* positions as well as the substituent at the C5 position of the thiazolidinone core on the prototropictautomerism and electronic structure. The initial 4-(*o*-,*m*-,*p*-hydroxyphenyl)amino-1,3-thiazol-2(5*H*)-ones were obtained utilizing nucleophilic replacement of isorhodanine with aminophenols [Komaritsa & Grishchuk, 1971; Kaminskyy *et al.*, 2015] in order to produce isomeric 5-dimethylaminomethylidene-4-(hydroxyphenyl)amino-1,3-thiazol-2(5*H*)-ones.The introduction of the 5-substituent was done *via* the Knoevenagel-type reaction with dimethylformamide dimethyl acetal (DMF-DMA) in dimethylformamide (Fig. 4.3.1).

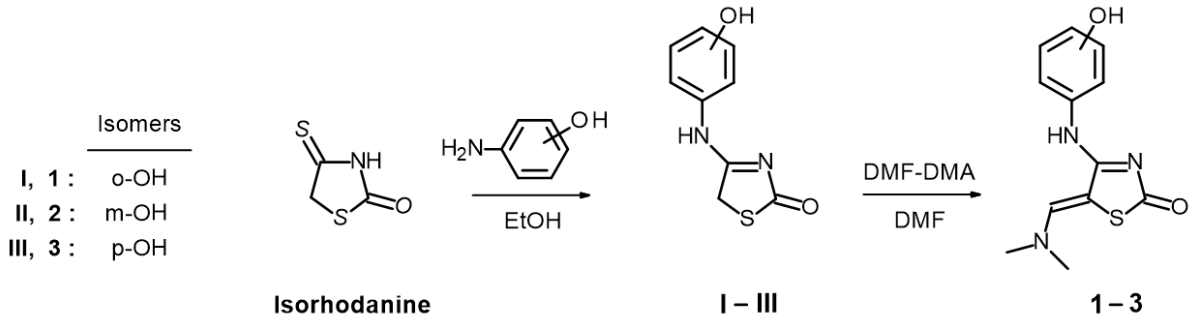


Fig. 4.3.1 Synthesis of 5-dimethylaminomethylidene-4-(*o*-,*m*-,*p*-hydroxyphenyl)amino-1,3-thiazol-2(5*H*)-ones (**3** – **5**).

It has been demonstrated through structural and spectroscopic studies that isomeric 5-dimethylaminomethylidene-4-(*o*-,*m*-,*p*-hydroxyphenyl)amino-1,3-thiazol-2(5*H*)-ones behave similarly to a related compound with an unsubstituted phenyl system (see publication D-1) by maintaining the amine tautomeric form (structure A, Fig. 4.3.2) and synperiplanar conformation of 4-hydroxyphenyl)amine residuein both the solid and liquid (DMSO) phases.

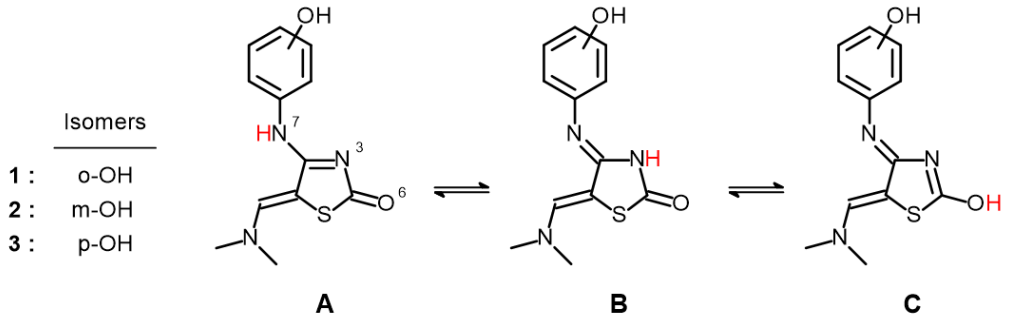


Fig. 4.3.2 Possible tautomeric structures of **3** – **5**.

In the solid phase studies, the position of the amidine H atom was determined from the difference Fourier map and refined freely with isotropic ADP parameters. The localization of the H atom at the N7 position was confirmed by hydrogen bonds N7–H7···O6^{i} (**3**) and N7–H7···O19 (**4·DMF** and **5·DMF**)) involving it, in which a carbonyl O atomplays the role of proton acceptor (Fig. 4.3.3).

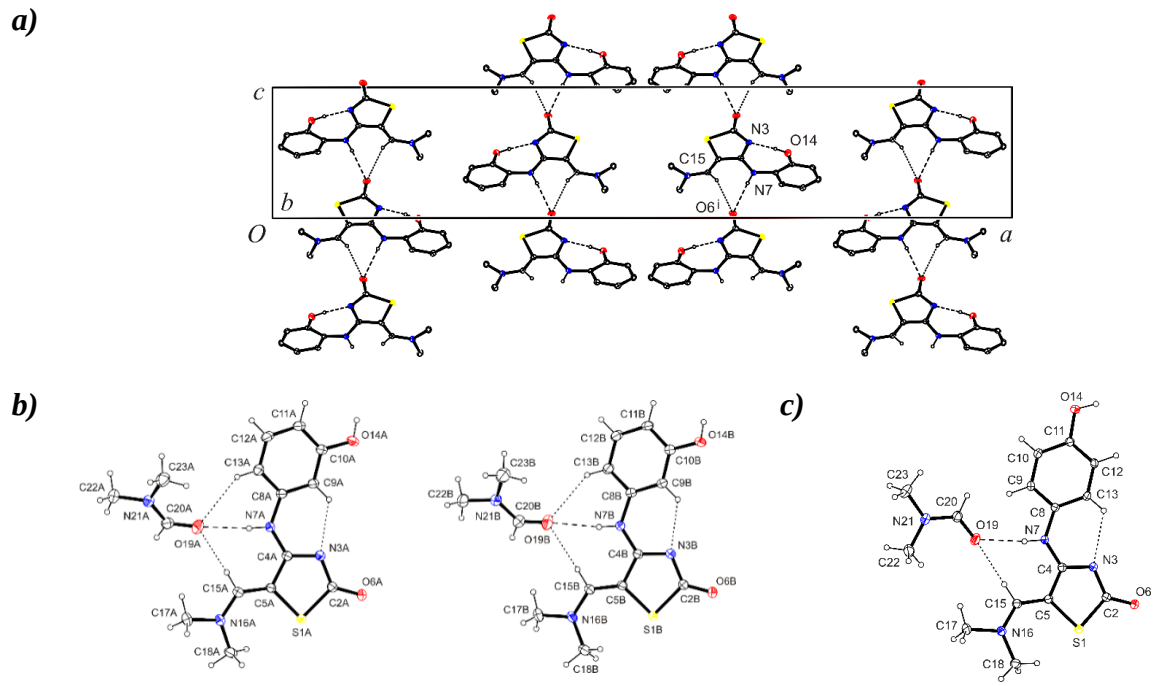


Fig. 4.3.3 ORTEP views showing (a) the molecules of **3** linked by the N7–H7···O6^{i} hydrogen bonds, (b) the independent molecules A and B of **4·DMF**, and (c) the molecules of **5·DMF** both linked by the N7–H7···O19 hydrogen bonds. Symmetry code: (i) 5/4–*x*,1/4+*y*,– 3/4+*z*.

Additional evidence for the presence of molecules in the amine tautomeric form in the crystals of **3 – 5** are intramolecular hydrogen bonds O14–H14···N3 (**3**) and C9(13)–H9(13)···N3 (**4·DMF** and **5·DMF**) (Fig. 4.3.3). The formation of thesecontacts is favored by the synperiplanar conformation of the (*o*-,*m*-,*p*-hydroxyphenyl)amine group in the molecules of **3** – **5** as well as the mutual spatial alignment of the phenyl and thiazolone systems[dihedral angles: 18.76(11)º (**3**), 11.35(4) / 16.05(4)º (**4**, molecules A / B), 31.57(3)º (**5**)].It is important to point out that dihedral angles found are increased in comparison with the analogous angle for related 5-dimethylaminomethylidene-4-phenylamino-2-thiazolinone, which was described in publication D-1. Despite this, however, the formation of the aforementioned intramolecular hydrogen bonding are still feasible.

As with the compounds in the publication D-1, observations relating to the amine tautomeric form in the crystals of **3** – **5** made by means of the X-ray crystallography were also demonstrated by the studies based on solid-state FT-IR analysis supported by theoretical calculations.

The X-ray analysis of **3** – **5** showed that the amine atom of N7 participates only slightly in the resonance effect with the carbonylimine group. The ylidene substituent at the C5 position

is the primary target of the coupling effect (Fig. 4.3.4). The finding supports earlier findings (see publication D-1) showing that the resonance effect mediated by the carbonylimine group is mainly directed toward the ylidene if it has electron-donor character. Otherwise, when the ylidene group is electro-withdrawing the resonance effect is mostly focused on the amine N7 atom.As in the related 5-dimethylaminomethylidene-4-phenylamino-2-thiazolinone, the reported low contribution of the N7 atom to the resonance effect accounts for the the strong upfield shift [δ = 9.08 ppm (**3**), 9.37 ppm (**4**) and 9.34 ppm (**5**)] of the signals for the amidine H atoms in the ^{1}H NMR spectrum of **3** – **5** when compared with the analogous signal (δ = 10.98 ppm) for 4-phenylamino-2-thiazolinone with an unsubstituted C5-methyl group.

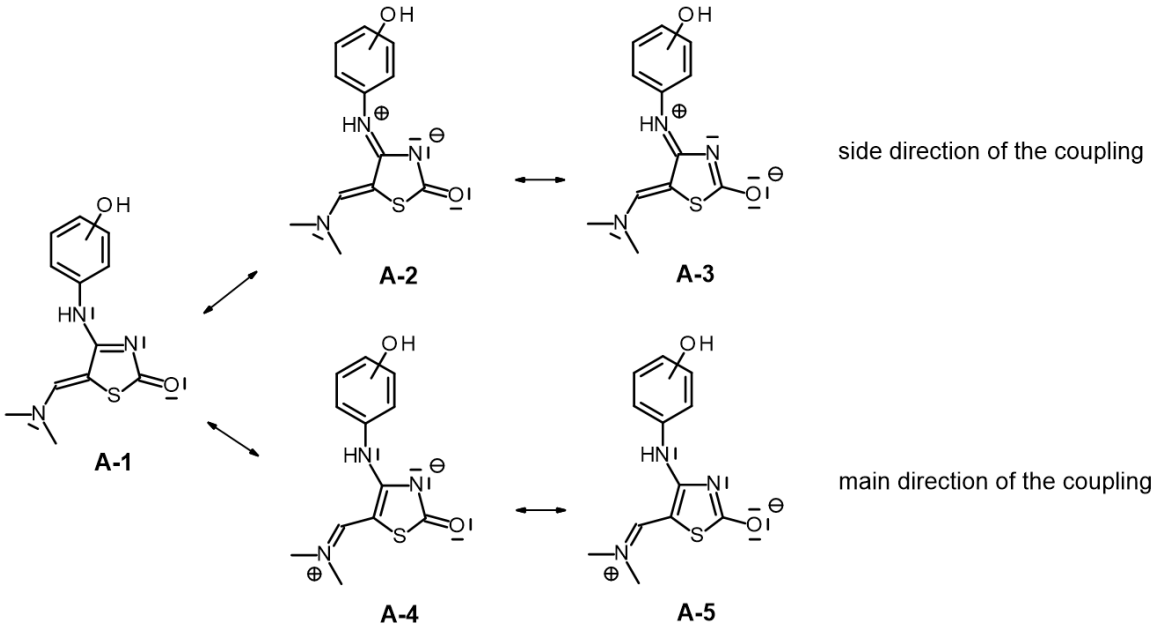
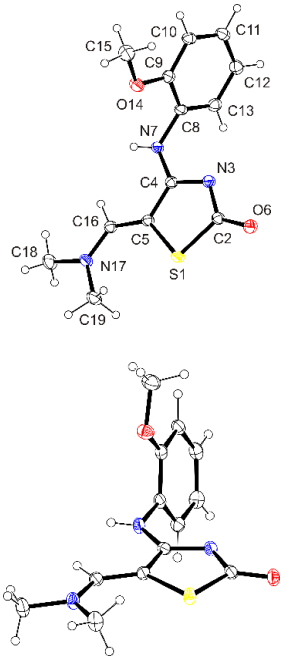


Fig. 4.3.4 Electron resonance in the molecules of **3**–**5**

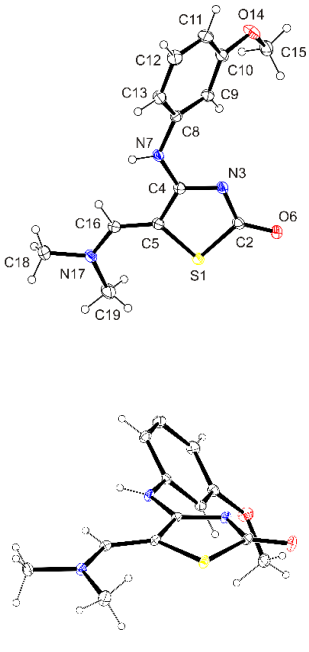
4.4 Proton tautomerism and stereoisomerism in isomeric 4-(methoxyphenyl)amino-1,3-thiazol-2(5*H*)-one derivatives: Synthesis, crystal structure and spectroscopic studies (publication D-3)

Publication D-3, highlights the synthesis and structural characterization of isomeric 5-dimethylaminomethylidene-4-phenylamino-1,3-thiazol-2(5*H*)-one derivatives (see publications D-1 and D-2) with a methoxy group substituted at the phenyl ring at the *ortho*, *meta*, and *para* positions. Compared to the hydroxy substituent, which is dual-function hydrogen bond donor and acceptor, described in article D-2, the sterically bulky methoxy group is only an acceptor of classical hydrogen bonds. The motivation for the present study was the expectation that intermolecular interactions, through their impact on the electronic structure and conformation of molecules in the crystalline state, should also influence such phenomena as tautomerism and stereoisomerism of the molecular core of interest.

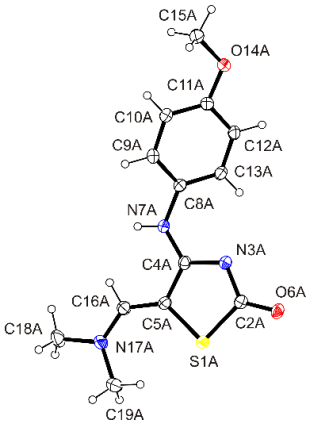
a)

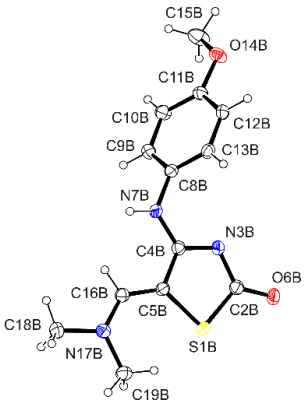


b)



c)





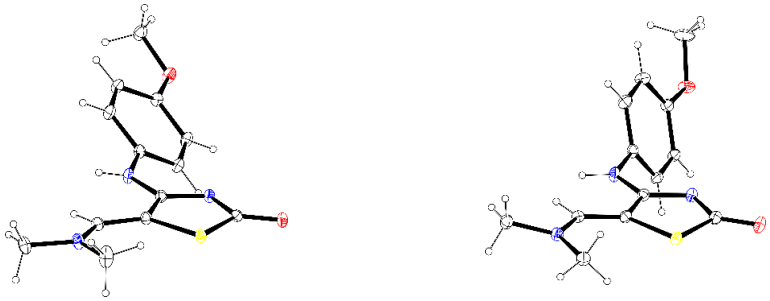


Fig. 4.4.1 Two views of (a) the molecule of **6**, (b) the molecule of **7**, and (c) the symmetry-independent molecules A and B of **8**.

The synthesis and crystallization of the title compounds were carried out as for the previously described analogs. X-ray and FT-IR (solid state) as well as ^{1}H and ^{13}C NMR (DMSO solution) spectroscopic studies were complemented with DFT theoretical calculations.

All isomeric compounds crystallize in the monoclinic system, in the $P2_1/c$ (**6**), $C2/c$ (**7**) and $P2_1/c$ (**8**) space groups. The unit cell of the crystals of **8** contains two symmetry-independent molecules differing by a small tilt of the 4-methoxyphenyl ring. As in the case of the hydroxy derivatives, all three methoxy-substituted molecules **6** – **8** were found to be the amino tautomers with the same synperiplanar conformation (Fig. D-3.1), while the dimethylaminomethylidene residue has the Z-configuration, as also observed for the molecules discussed in comments D-1 and D-2. The location of the amidine H atoms based on the difference Fourier maps, was successfully corroborated by intermolecular hydrogen bonds. Specifically, there are hydrogen bonds between the N7-H donor and the O6 acceptor of the carbonyl group of adjacent molecules for **6** – **8**, and a non-classical hydrogen bond between the N3 acceptor and the methyl-group donor of an adjacent molecule for **8**, clearly indicating the amino form of all three compounds, as illustrated by a crystal-packing diagram for **7** (Fig. D-3.2).

In general, the incorporation of the methoxy group had a major impact on the planarity of the molecule when compared with hydroxy analogs, but not on the tautomeric form or conformational arrangement at the N7 nitrogen. The bulky methoxy group causes a significant rotation of the phenyl ring relative to the thiazolidinone cycle, which does not seem to be correlated with the position of the substituent, as the largest torsion angles are observed for the *ortho* (**6**) and *para* (**8**) derivatives. Such a deformation of molecular planarity precludes intramolecular interactions between the N3 nitrogen of the heterocycle with the atoms (H atom or substituent) at the *ortho* position of the arene ring, which was possible for the much more planar hydroxy derivatives. The participation of the methoxy group in lattice formation was observed for compounds **6** (unconventional C–H$\cdots\pi$ bonds with both the phenyl and thiazolidinone rings) and **8** (unconventional C–H$\cdots$S bond accepted by the sulfur atom of the thiazolidinone ring). The short H…H contact between the N7-H group and the C5-H methylidene bridge, is a systematically present, characteristic feature of this class of compounds.

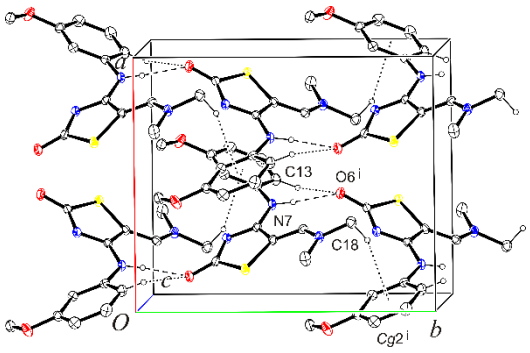


Fig. 4.4.2 Hydrogen-bonding and C–H···*Cg* interactions in the crystal of **7**.

All the above observations suggest a high stability of the amine tautomeric form, regardless of the position of the -OCH_3 group at the arene ring, as well as high a stability of the synperiplanar conformation of the methoxyphenylamine residue and the *Z*-configuration of the dimethylaminomethylidene moiety of all three isomeric compounds, both in the liquid (DMSO solutions) and solid phases.

By DFT calculations, it was also discovered that the dimethylaminomethylidene fragment competitively reduces the contribution of the exocyclic N7 amine nitrogen atom to the electron coupling effect with the carbonylimino group, which was previously observed in the molecules described in publications D-1 and D-2.

4.5 Proton tautomerism and stereoisomerism in 5-[(dimethylamino)methylidene]-4-[3/4-(trifluoromethylphenyl)amino]-1,3-thiazol-2(5*H*)-ones: synthesis, crystal structure and spectroscopic studies (publication D-4)

This work elucidates the structures of 5-dimethylaminomethylidene-4-phenylamino-thiazolidinones with trifluoromethyl group substituted at the phenyl ring. Having accumulated in the previous papers the data about the influence of substituents with positive mesomeric effects, I turned in paper D-4 my attention to a group having strictly negative (electron withdrawing) electronic effect to test its impact on the tautomerism and stereoisomerism of the title compounds. The substituent of choice was -CF_3, which according to literature [Minkin *et al.*, 2000]) should have a pure negative electronic effect on the aromatic core.

The synthetic path to the title compounds was adapted from Komaritsa and Pindela [Komaritsa & Grishchuk, 1971; Pindela, 2020], where the crucial and most difficult step was the reaction of isorhodanine with trifluoromethylanilines. Unfortunately, all attempts to obtain the third, *ortho*, isomer under these conditions were unsuccessful. It can be, at least partially, explained by the reduced nucleophilicity of the amino group in reaction with isorhodanine.

As in the previous studies, set of methods used to investigate the problems of tautomerism and stereoisomerism included single crystal X-ray diffraction, NMR and FT-IR spectroscopy, as well as DFT calculations. The crystals of both **9** *(meta)* and **10** *(para)* are monoclinic $P2_1/c$, with one molecule in the asymmetric unit. Both compounds turned hard to be crystallized. Crystals of **9** form difficult to separate conglomerates and have a disordered trifluromethyl group. Crystals of **10** are psedomerohedral twins with a twin fraction of 22.00(19)% and, moreover, reveal positional disorder of whole molecules by the action of a pseudo twofold axis (Fig. 4.5.1). As for previously discussed cases, the positions of hydrogen atoms were located from difference Fourier maps and additionally confirmed by intermolecular hydrogen bonds.

To my surprise, both compounds were again found to exist as amino tautomers, both in the solid and liquid phase. The crystal structures revealed the synperiplanar conformation of the (trifluoromethylphenyl)amine residue and *Z*-configuration of the dimethoxyaminomethylidene moiety. Again, any hypothetical traces of the imino tautomer were beyond the detection level of the methods, both in the solid state and in DMSO solution. The repeatedly observed intermolecular hydrogen bonds between the N7-H donor and the O6 carbonyl acceptor of an adjacent molecule, confirmed these findings. In contrast to the methoxy derivatives (see publication D-3), an intramolecular contact between the N3 atom and the proximal proton of the phenyl ring tends to stabilize the nearly coplanar arrangement of the ring systems. Theoretical simulations were in full agreement with the experimental data.

We can, therefore, come to the conclusion about the exceptional stability of the amino form and synperiplanar conformation of 4-phenylaminothiazolidinones. This is in stark contrast to the experimental reports about the tautomeric forms of the isomeric 2-phenylaminothiazolidinones [Kowiel, 2015], where the presence of an electron-withdrawing group in the phenyl ring, especially proximal to the amidine system, promoted a shift of the tautomeric equilibrium to the imino form and promoted the *E*-configuration of the phenylamino moiety.

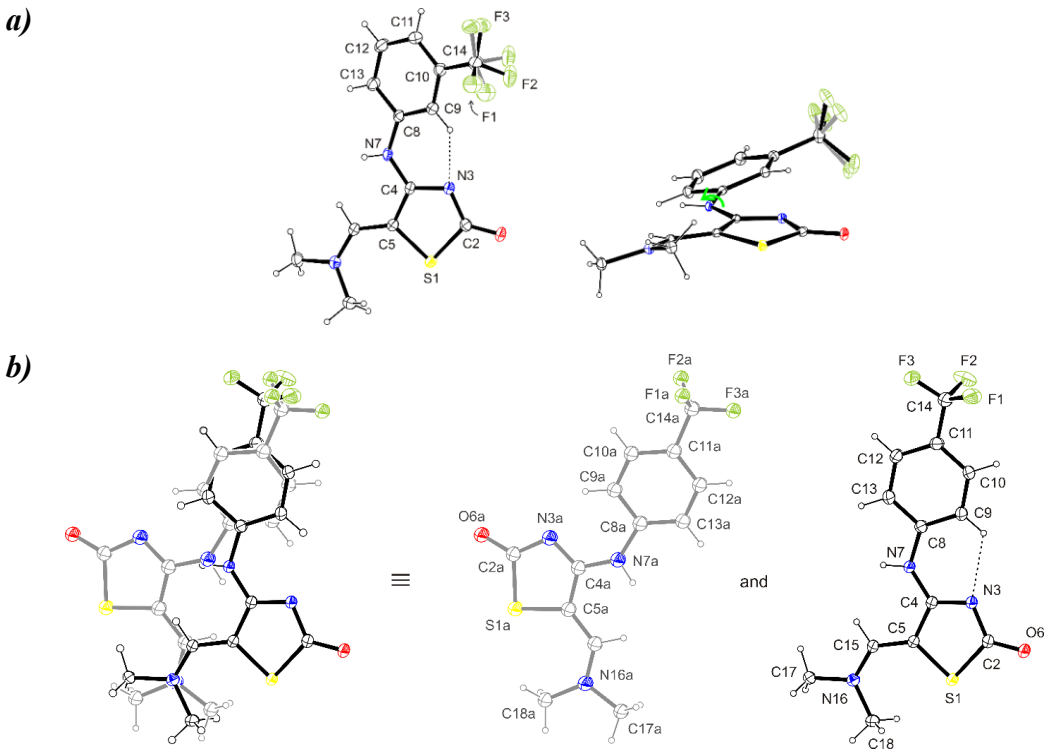


Fig. 4.5.1 The molecular structures of **9** and **10** found in their crystals. (a) The molecule of **9** is presented in two views and (b) the molecule of **10** is split into two alternative copies (black is the major form; gray is the minor form).

4.6 Synthesis, structure and evaluation of anticancer activity of 4-amino-1,3-thiazolinone/ pyrazoline hybrids (publication D-5)

Molecular hybridization, i.e. combining in one molecule two or more pharmacofore scaffolds, is a wide-spread approach to the design of diversified small-molecule libraries. Thiazolidinones are no exception and there are many reports of lead compounds bearing this core together with other heterocyclic scaffolds, fragments of naturally occurring compounds, molecular assemblies found in already known drugs, etc. (see Introduction section). This strategy can be applied (i) in structure-based drug design, (ii) for the discovery of molecules with affinity for multiple biological targets, (iii) to improve the selectivity and/or modulate bioavailability of already known active compounds. For instance, the connection in one molecule of 2-aminothiazolidinone and pyrazoline cycles has led to the identification of compounds with excellent anticancer potential [Havrylyuk *et al.*, 2009]. Thus, it was reasonable to focus in my structural studies also on the biological aspect and to create previously unknown isomeric compounds based on the 4-aminothiazolidinone scaffold, with an attached pyrazoline moiety and to investigate the impact of these structural modifications on tautomerism and stereoisomerism of the molecules.

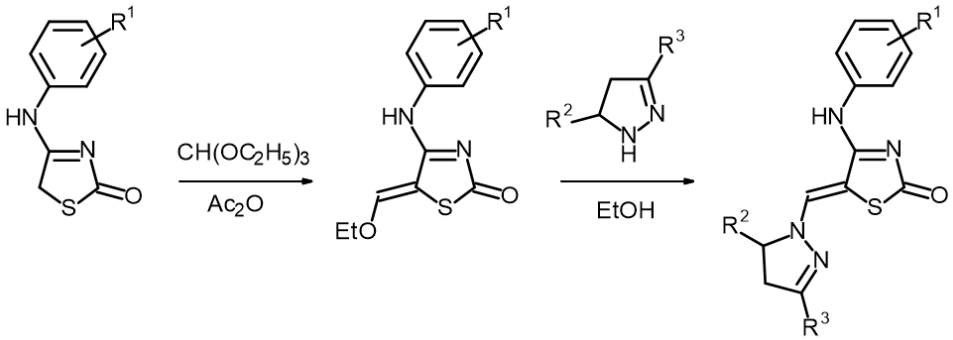


Fig. 4.6.1 Synthesis of 5-ethoxymethylidene-4-arylamino-1,3-thiazoline-2-ones and 5-[3,5-di(het)aryl-4,5-dihydro-1*H*-pyrazoline-1-yl)methylene]-4-arylamino-1,3-thiazoline-2-ones: **11**, R^1 = 4-AcO, R^2 = 4-F-C_6H_4, R^3 = 4-MeO-C_6H_4; **12**, R^1 = Cl, R^2 = thien-2-yl, R^3 = 4-Cl-C_6H_4; **13**, R^1 = F, R^2 = 2,4-F_2-C_6H_3, R^3 = 3-MeO-C_6H_4; **14**, R^1 = F, R^2 = 4-F-C_6H_4, R^3 = 4-MeO-C_6H_4; **15**, R^1 = 4-Cl, R^2 = 2,4-F_2-C_6H_3, R^3 = 3-MeO-C_6H_4; **16**, R^1 = AcO, R^2 = R^3 = 4-F-C_6H_4; **17**, R^1 = 4-Cl, R^2 = 4-F-C_6H_4, R^3 = 4-Cl-C_6H_4; **18**, R^1 = 4-F, R^2 = 4-F-C_6H_4, R^3 = 4-Cl-C_6H_4; **19**, R^1 = 4-Cl, R^2 = R^3 = 4-F-C_6H_4; **20**, R^1 = 4-F, R^2 = thien-2-yl, R^3 = 4-Cl-C_6H_4.

My synthetic strategy to create such hybrid molecules was based on the reaction of 4-arylaminothiazolidinones with triethylorthoformate in the presence of acetic anhydride (Fig. 4.6.1), with subsequent nucleophilic substitution of the ethoxy group with a freshly prepared diaryl(hetaryl)pyrazoline derivative. It is of note that the derivative with a hydroxy group at the phenyl ring was acetylated under these conditions. As a result, I prepared and characterized 10 new

compounds. For one of them (compound **11**), which is a representative of the entire set of the created library, I applied single-crystal X-ray structural analysis, revealing a non-centrosymmetric space group *Pca*2_1 of the crystals, with two symmetry-independent molecules, labeled A and B in Fig. 4.6.2. The molecules are almost identical and differ only by the angular arrangement of the acetoxy group at the phenylamino fragment. A thorough structural analysis revealed several characteristic features of 4-phenylaminothiazolidinones, namely (i) a non-classical intramolecular N3···H–C(*ortho*) hydrogen bond, formed despite a slight deviation of the phenyl and thiazolidinone rings from strict coplanarity; (ii) short H···H contacts between the H atoms at the N7 amino nitrogen and C15 methylidene group.

The title compounds, like their simpler analogs discussed previously [Pyrih *et al.*, 2023a, 2023b, 2023c, 2024], can be found theoretically in three tautomeric forms. However, as in all the previous cases, they were found experimentally to exist as the amino tautomers with synperiplanar conformation of the phenyl ring at the N7 nitrogen atom. The absence of additional peaks in the ^{1}H NMR spectra that may indicate other tautomeric forms in all the studied compounds allows one to assume that only one tautomeric form is also present in DMSO solutions. Interestingly, the resonance effects observed in the title pyrazoline-containing molecules (manifested as an elongations of the double bond and shortening of the single bond within the carbonylimino and aminomethylidene moieties, as well as the upfield shifts of the protons at the N7 nitrogen atom in the ^{1}H NMR spectra) are in excellent agreement with the data reported previously for compounds with the dimethylamino group [Pyrih *et al.*, 2023a, 2023b, 2023c, 2024].

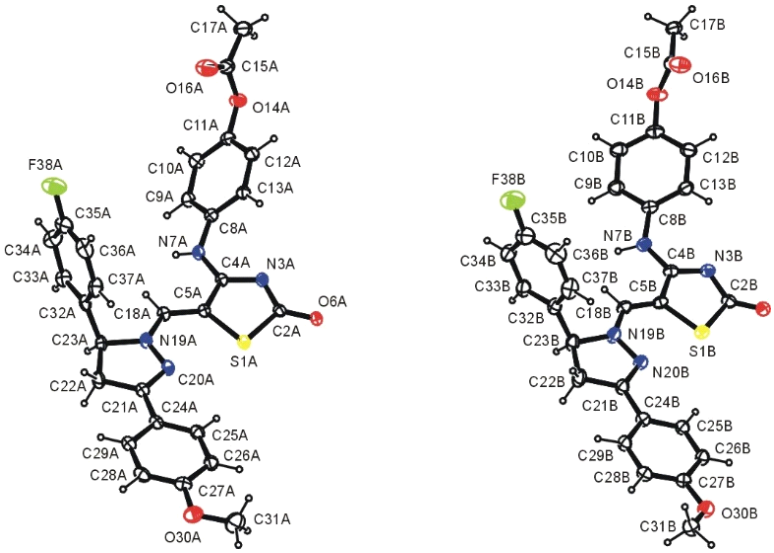


Fig. 4.6.2 ORTEP view of the two symmetry-independent molecules of **11**.

The biological activity of compounds **11**, **14** – **19** was evaluated in collaboration with the National Cancer Institute (NCI, Bethesda, USA) according to the NCI protocol of the Drug Evaluation Branch [Monks et al., 1991; Boyd & Paull, 1995; Shoemaker, 2006]. The biological experiments with 60 cancer cell lines revealed that the tested compounds possess rather moderate anticancer activity (Table 4.6.1), which may be, at least partly, explained by their low solubility in

water. The highest growth inhibition rates were observed for the derivatives **17** and **18**. An interesting feature of the tested compounds is their selective action on the renal cancer cell line UO-31.

Table 4.6.1 Anticancer screening data in concentration of 10 μM

Comp.	**60 cell lines assaying 1 dose of 10 μM concentration**			
	mean growth, %	range of growth, %	most sensitive cell lines	
			cell line (type of cancer)	GP, %
11	99.35	82.67 to 117.92	SR (Leukemia) UO-31 (Renal Cancer)	85.60 82.67
14	97.13	73.41 to 106.10	EKVX (Non-Small Cell Lung Cancer) UO-31 (Renal Cancer)	82.17 73.41
15	97.65	60.45 to 129.66	LOX IMVI (Melanoma) UACC-62 (Melanoma) UO-31 (Renal Cancer)	71.75 71.90 60.45
16	98.55	75.35 to 130.56	NCI-H522 (Non-Small Cell Lung Cancer) UACC-62 (Melanoma) UO-31 (Renal Cancer)	77.73 75.35 81.26
17	89.19	59.97 to 112.83	K-562 (Leukemia) RPMI-8226 (Leukemia) NCI-H522 (Non-Small Cell Lung Cancer) HCT-116 (Colon Cancer) SNB-75 (CNS Cancer) SK-MEL-5 (Melanoma) UACC-62 (Melanoma) CAKI-1 (Renal Cancer) UO-31 (Renal Cancer)	73.14 74.54 77.19 78.72 74.58 70.14 69.46 77.20 59.97
18	90.93	64.72 to 107.46	K-562 (Leukemia) RPMI-8226 (Leukemia) EKVX (Non-Small Cell Lung Cancer) UO-31 (Renal Cancer) T-47D (Breast Cancer)	79.60 69.99 77.82 64.72 74.40
19	96.43	70.98 to 106.98	EKVX (Non-Small Cell Lung Cancer) UO-31 (Renal Cancer)	82.76 70.98

GP – growth percent of treated cells compared to untreated control ones.

5. SUMMARY

The Ph.D. thesis presents the synthesis and structural analysis of **20 derivatives** of the 1,3-thiazolidinone core of potential pharmacological interest. The results of the structural analyses by X-ray crystallography and FTIR spectroscopy (for solid phase samples), as well as by ^{1}H and ^{13}C NMR (for DMSO solutions), accompanied by quantum chemical simulations, were presented in five publications **D-1 to D-5**, complemented by an introductory paper **D-0**. After careful structural analysis, the newly synthesized derivatives of 4-amino-1,3-thiazol-2(5*H*)-one, functionalized by:

– 4-phenylamine and 5-dimethylaminomethylidene substituents (**1**), and by 4-phenylamine and 5-methoxycarbonylmethylidene substituents (**2**),
– electron-donating groups (-OH: **3**–**5**; -OCH_3: **6**–**8**) around the arene ring at the *ortho*, *meta* and *para* positions,
– electron-withdrawing group (-CF_3: **9**, **10**) around the arene ring at the *meta* and *para* positions,
– 4-phenylamine and heterocyclic 1*H*-pyrazoline-1-yl-methylidene moiety at the C5 position (**11**–**20**)

were the basis for the following **conclusions**:

- The compounds crystalize mainly in the monoclinic system (**1-I**, **1-II**, **2**, **4-10**), rarely in the orthorombic system (**3**, **11**). Nine crystals are centrosymmetric, space groups $P2_1/c$: **2**, **4-6**, **8-10**; $P2_1/n$: **1-II**; $C2/c$: **7**, three crystals are non-centrosymmetric, space groups *Cc*:**1-I**; *Fdd*2: **3**; $Pca2_1$: **11**. The crystals of two compounds are formed as solvates with one molecule of dimethylformamide (**4**·**DMF**, **5**·**DMF**);
- All the explored compounds **1-20** exist as amino tautomers in both the solid and liquid (DMSO solution) phase, likewise the unsubstituted parent 4-amino-1,3-thiazol-2(5*H*)-one, meaning that neither the phenyl ring, alone or with substituents, nor electron-donating or electron-withdrawing C5 substituents, can influence the tautomeric equilibrium;
- Electron-donating C5 substituents at the thiazolidinone system have a significant contribution to the electron resonance effect with the carbonylimino fragment of the thiazolidinone system, reshaping the electronic landscape of the molecules;
- In the solid and liquid phases, the phenylamino moiety was adopts only the synperiplanar conformation, as corroborated by quantum chemical DFT calculations;
- The new compounds are interesting scaffolds as future antiproliferative agents.

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8. SUPPLEMENTARY MATERIALS

Table S1 Refcodes of 2-amino-1,3-thiazol-4(5*H*)-one derivatives found in Cambridge Structural Database, Version 5.43 (Groom *et al.*, 2016)

AKUXAH, ATAZAC10, BOQVEL, EGAQIP, EHOBOV, EHOBUB, EKELEL, FIVPIJ, FIVPIJ01, FOWQOY, GECXEU, IHUFAS, IHUKIH, IMPTHA01, IMPTHA12, IMTAZO01, IMTAZO02, INMTZO, JOBGOW, KODJUJ02, KUKZUM, LOQBIE, LOQBOK, PACPIU, PATAZO, PTHAZO10, SALYOT, SINQOW, SINQUC, TEBDAH, ULACAM, VELBEU, WOSMAS, XAXHAI, XULHOD, YUQCAP

Table S2 Refcodes of 2-imino-1,3-thiazol-4(5*H*)-one derivatives found in Cambridge Structural Database, Version 5.43 (Groom *et al.*, 2016)

GACXOZ, HEGLUC, HEGMAJ, HEGMEN, HEGMIR, HEGMOX, IMTAZO. LAFJAG, RALVEI, SISCAB, VAMPUW, ZORYUD

Table S3 Interatomic distances C2–N3 and C2–N6 in molecules of 2-amino-1,3-thiazolin-4-ones occurring in the amine tautomeric form ($R < 7.5\%$)

	Refcodes	**C2–N3 (Å)**	**C2–N6 (Å)**
1.	AWUPEO	1.311	1.325
2.	BAHGOI	1.307	1.327
3.	BOQVEL	1.324	1.314
4.	CELLAI	1.315	1.327
5.	DEWRIJ	1.314	1.333
6.	EMOHAS	1.308	1.338
7.	ERATUM	1.310	1.326
8.	FIVPIJ	1.318	1.318
9.	FIVPIJ01	1.316	1.314
10.	GACXUF	1.324	1.326
11.	GECXEU	1.328	1.316
12.	GINYEJ	1.310	1.321
13.	GIXTOZ	1.315	1.318
14.	HIDJIO	1.335	1.316
15.	HUDLUP	1.310	1.327
16.	IHUKIH	1.326	1.325
17.	IREXEK	1.316	1.320
18.	IREXIO	1.311	1.320
19.	IREXOU	1.324	1.322
20.	IREYAH	1.309	1.322
21.	IREYEL	1.318	1.321
22.	IREYIP	1.311	1.320
23.	IREYOV	1.307	1.326
24.	IREYOV	1.314	1.326
25.	IREYUB	1.314	1.317
26.	IREZAI	1.305	1.324

27.	IREZEM	1.312	1.319
28.	JOJMED	1.301	1.334
29.	KUKZUM	1.327	1.317
30.	KUKZUM	1.317	1.320
31.	KUQKUD	1.322	1.309
32.	LAGMEO	1.308	1.324
33.	LAGMEO	1.312	1.324
34.	LENTEF	1.300	1.328
35.	LEQZIS	1.316	1.336
36.	LEQZIS01	1.306	1.319
37.	LOQBOK	1.318	1.328
38.	PTHAZO10	1.322	1.334
39.	PTHAZO10	1.315	1.344
40.	REZCIJ	1.312	1.329
41.	SINQOW	1.319	1.309
42.	SINQOW	1.319	1.311
43.	SINQUC	1.322	1.309
44.	SINQUC	1.316	1.316
45.	TEBDAH	1.320	1.321
46.	UBUYEZ	1.320	1.327
47.	UWOJIB	1.310	1.324
48.	UYIPIC	1.313	1.325
49.	VELBEU	1.322	1.339
50.	XAXHAI	1.330	1.324
51.	XULHOD	1.333	1.319
52.	XULHOD	1.312	1.331
53.	YAGFET	1.318	1.326
54.	IREYUB01	1.323	1.331
55.	MEMSUW	1.319	1.325
56.	MEMTAD	1.316	1.317
Mean value		**1.316(1)**	**1.323(1)**

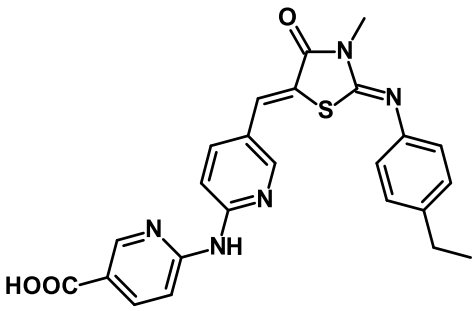
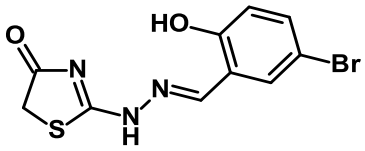
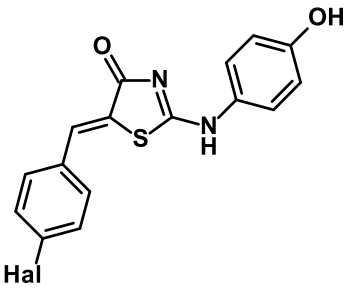
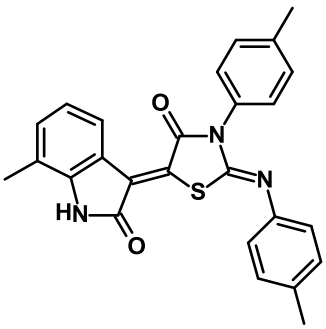
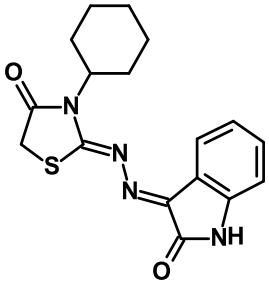
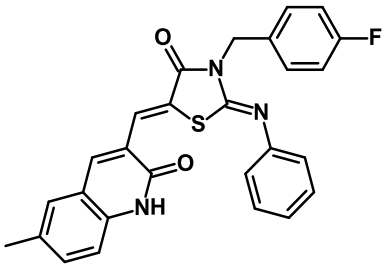
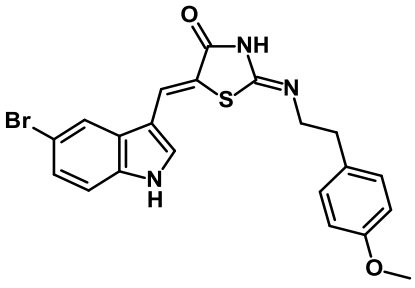
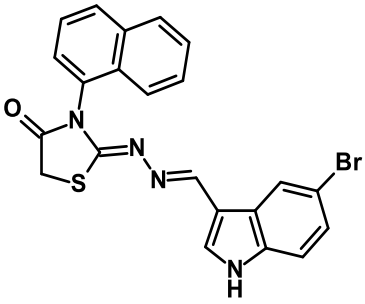
Table S4 The reference lengths of double and single Csp^2–N bonds obtained based on structures deposited in Cambridge Structural Database, version 5.43.

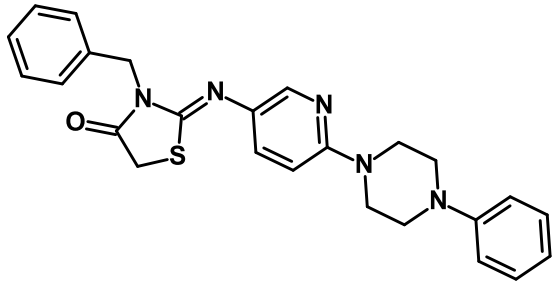
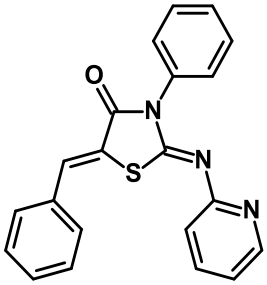
	Refcodes	**C=N (Å)**	**Csp^2–N (Å)**
1.	EHITZO	1.282	1.387
2.	FOTQEM	1.269	1.380
3.	HEGMAJ	1.281	1.362
4.	HEGMAJ	1.268	1.372
5.	HEGMEN	1.282	1.381
6.	HEGMIR	1.296	1.363
7.	HEGMOX	1.277	1.369
8.	HEGMOX	1.284	1.371
9.	IKIVIJ	1.291	1.360
10.	JOCKIY	1.280	1.384
11.	JOCKOE	1.288	1.370
12.	JOMCOF	1.271	1.383

13.	JOMKOO	1.271	1.379
14.	LAFJAG	1.297	1.373
15.	NEBHOU	1.283	1.367
16.	NOYYOS	1.277	1.356
17.	OCAGEI	1.272	1.371
18.	POSSOH	1.271	1.382
19.	QAJSEC	1.280	1.368
20.	RALVEI	1.280	1.374
21.	RALVEI	1.266	1.382
22.	ROMXUN	1.278	1.367
23.	SISCAB	1.270	1.373
24.	TIQTIZ	1.285	1.370
25.	TIQTIZ	1.276	1.381
26.	ULACEQ	1.260	1.390
27.	VAMPUW	1.292	1.380
28.	WAGVUY	1.286	1.369
29.	WOGGOQ	1.288	1.380
30.	XOKLAN	1.281	1.368
31.	XOKLER	1.281	1.371
32.	XUBYOK	1.279	1.379
33.	YIKXOI	1.278	1.374
34.	YIKXOI	1.270	1.378
Mean values		**1.279(1)**	**1.374(1)**

Table S5 Refcodes of 4-amino-1,3-thiazol-2(5*H*)-one derivatives found in Cambridge Struuctral Database, version 5.43 (Groom *et al.*, 2016)

CUBKIU, EYESUY, KERDAP, KERDIX, LAFHUY, MURCOU

 **I**	 **II**
 **III**	 **IV**
 **V**	 **VI**
 **VII**	 **VIII**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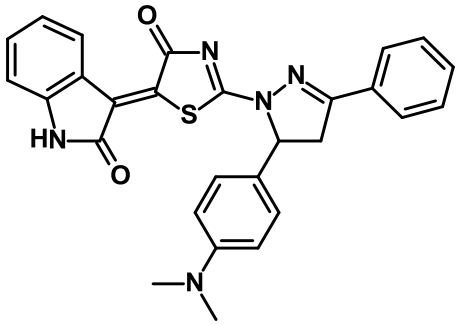	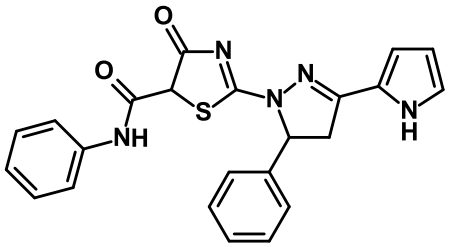
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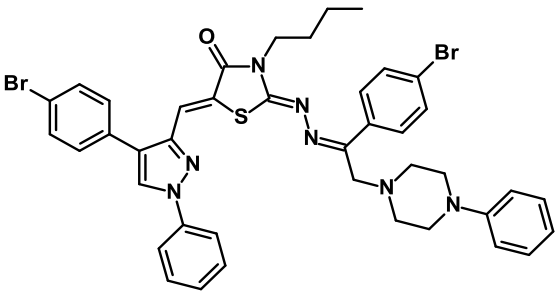	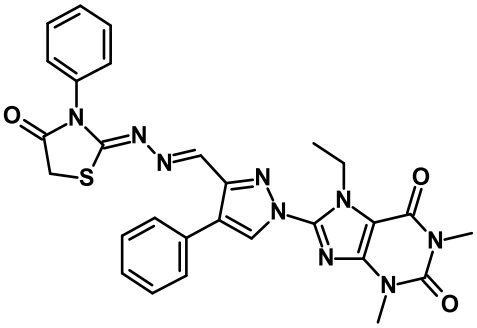
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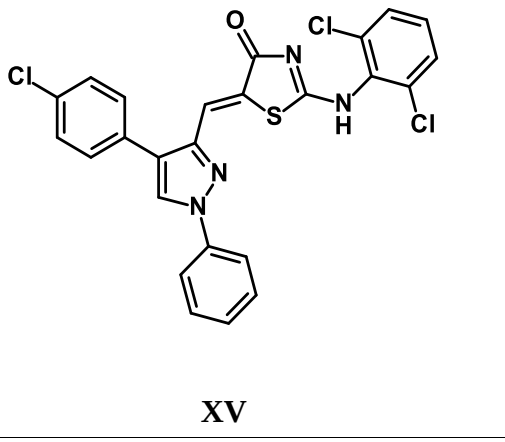	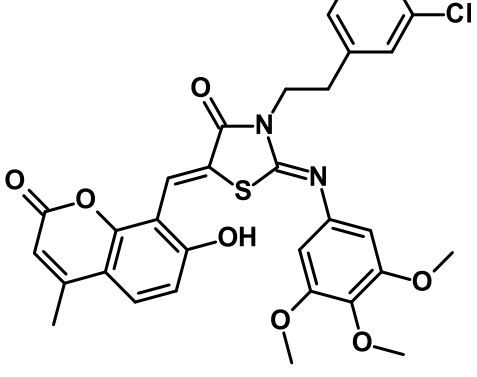
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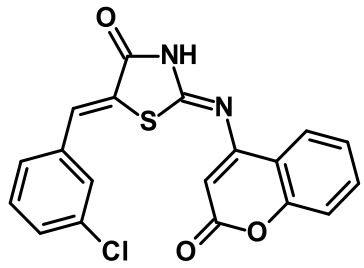	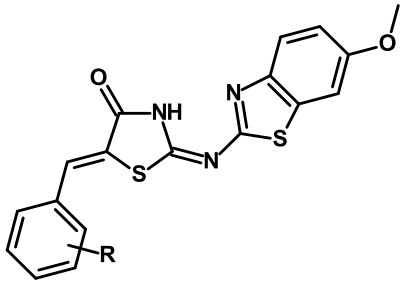
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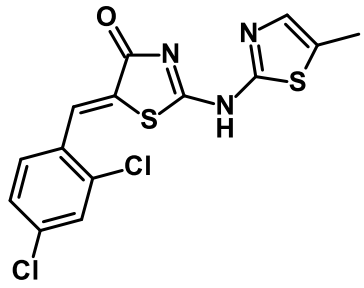	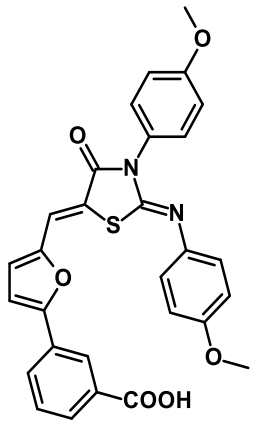
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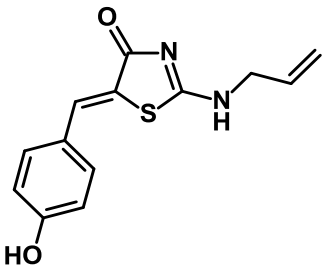	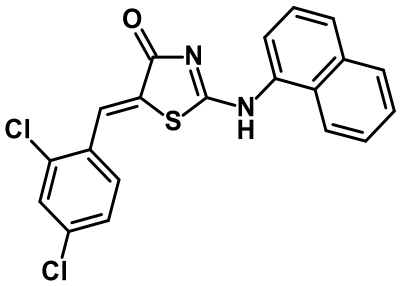
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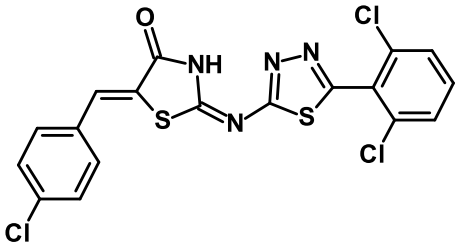	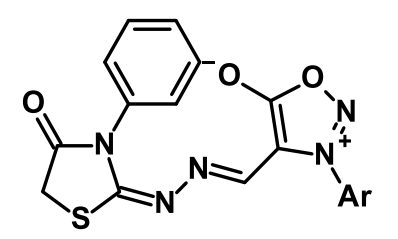
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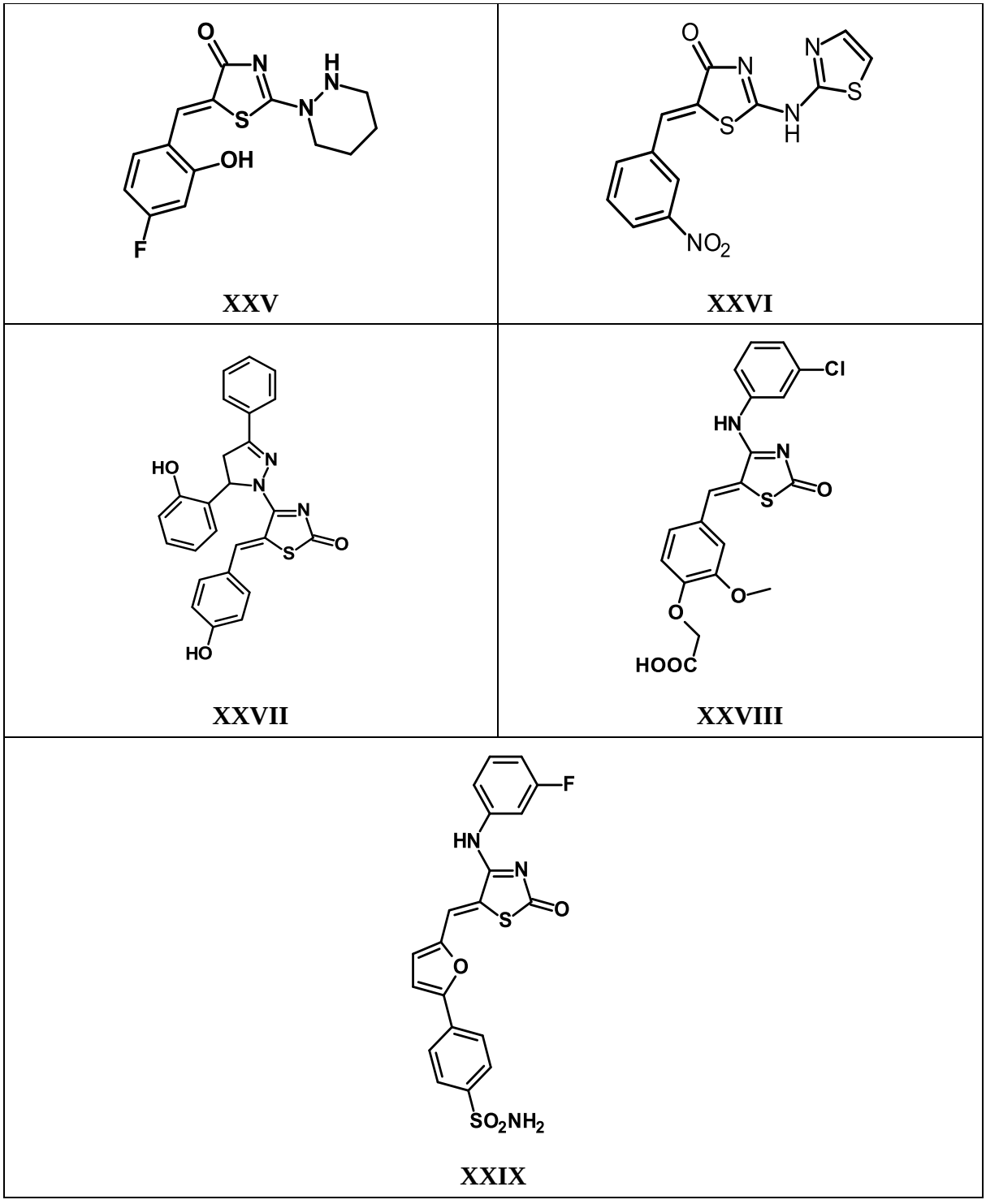

Fig. S1 Structural formulas of aminothiazolidinones discussed in the Introduction section.