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Hassan, Alaa A.

Springer
2022-06-09

Hassan, A A, Aly, A A, Ramadan, M, Mohamed, N K, Youssif, B G M, Gomaa, H A M, Braese, S, Nieger, M & El-Aal, A S A 2022, 'Synthesis of bis-thiohydantoin derivatives as an antiproliferative agents targeting EGFR inhibitory pathway', Molecular Diversity. https://doi.org/10.1007/s11030-023-1

http://hdl.handle.net/10138/576806
10.1007/s11030-023-10653-3

acceptedVersion

Synthesis of bis-thiohydantoin derivatives as an antiproliferative agents targeting EGFR inhibitory pathway

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Abstract

(*R*)/(*S*)-the two enantiomers of 3-substituted-1-[2-(5)-3-substituted-4-benzyl-5-oxo-4-phenyl-2-thioxoimidazolidin-1-yl]ethyl/propyl-5-benzyl-5-phenyl-2-thioxoimidazolidin-4-ones were formed during the diastereoselective reaction between *N*,*N*``-1,ω-alkanediylbis[*N*`-organylthiourea] derivatives and 2,3-diphenylcyclopropenone in refluxing ethanol. The structures of the isolated compounds were confirmed by NMR, IR, mass spectra and elemental analyses. Moreover, single-crystal X-ray structure analysis was also used to elucidate the structure of the isolated compounds. The mechanism describes the reaction was also discussed. The tested compounds showed EGFR inhibitory activity with IC_{50} values ranging from 90 nM to 178 nM in comparison to the erlotinib as a reference with IC_{50} value of 70 nM. Compound **4c** (R = allyl, n = 3) was found as the most potent antiproliferative, had the highest inhibitory effect on EGFR with an IC_{50} value of 90 nM, compared to erlotinib's IC_{50} value of 70 nM. The second and third-most active compounds were **4e** (R = phenyl, n = 3) and **4d** (R = ethyl, n = 3) and with IC_{50} values of 107 nM and 128 nM. These findings imply that the compounds tested had a significant antiproliferative effect as well as the ability to act as an EGFR inhibitor. Docking studies showed that compound **4c** showed high affinity to EGFR based on its docking score (S; kcal/mol) within five test compounds.

***Keywords*:** Thiourea, 2,3-diphenyl-cyclopropenone, Thioxoimidazolidin-4-ones, Antiproliferative, Docking.

1. INTRODUCTION

Several natural and synthesized heterocyclic compounds have been investigated as possible pharmacological pro-drugs throughout the last few decades [1]. Because of their presence in natural and therapeutically useful chemicals, 2-thiohydantoins (2-thioxo-imidazolidine-4-one), the sulfur analogues of hydantoin, have been recognized as fascinating chemical scaffolds (**Fig. 1**). Structural modifications of the 2-thiohydantoin ring yield compounds with a diverse range of pharmacological and biological effects, with anticancer activity topping the list [2-6]. The 2-thiohydantoin ring has also been found in the structures of various natural compounds, including Enzalutamide, which has been licensed by the FDA as a treatment for castration-resistant prostate cancer (**Fig. 1**) [7-9]. Because of the therapeutic importance of hydantoin/2-thiohydantoin-based molecules, the synthesis of a novel class of substituted-hydantoins/2-thiohydantoins has received a lot of interest in the last few decades [10-13].

Aziz Mohammadi et al described a novel series of hydantoin-chromene hybrids (**I**, **Fig. 1**) with antiproliferative action [14]. The compounds were tested for antiproliferative efficacy against a panel of five cancer cell lines. In comparison to the reference drug cisplatin, the hydantoin-chromene hybrids demonstrated mild to good anticancer activity. Alanazi et al. developed a series of 3-aminoalkyl/benzyl phenytoins **II** and 3-(4-piperidino/piperazino/morpholino)phenacyl phenytoins **III**, **Fig. 1** [15]. These hydantoin derivatives were found to exhibit anticancer action by decreasing epidermal growth factor receptor (EGFR) kinase activity. Interestingly, compound (**IIIa**) shown promising antiproliferative action. Bae et al. identified 3-aryl 5-benzylidene-1-methyl-2-thiohydantoins **IV** as new NADPH-oxidase (NOXs) inhibitors [16]. In that investigation, the synthesised compounds were tested for NOX1 and NOX4 inhibitory activities. **IV** demonstrated good inhibitory action.

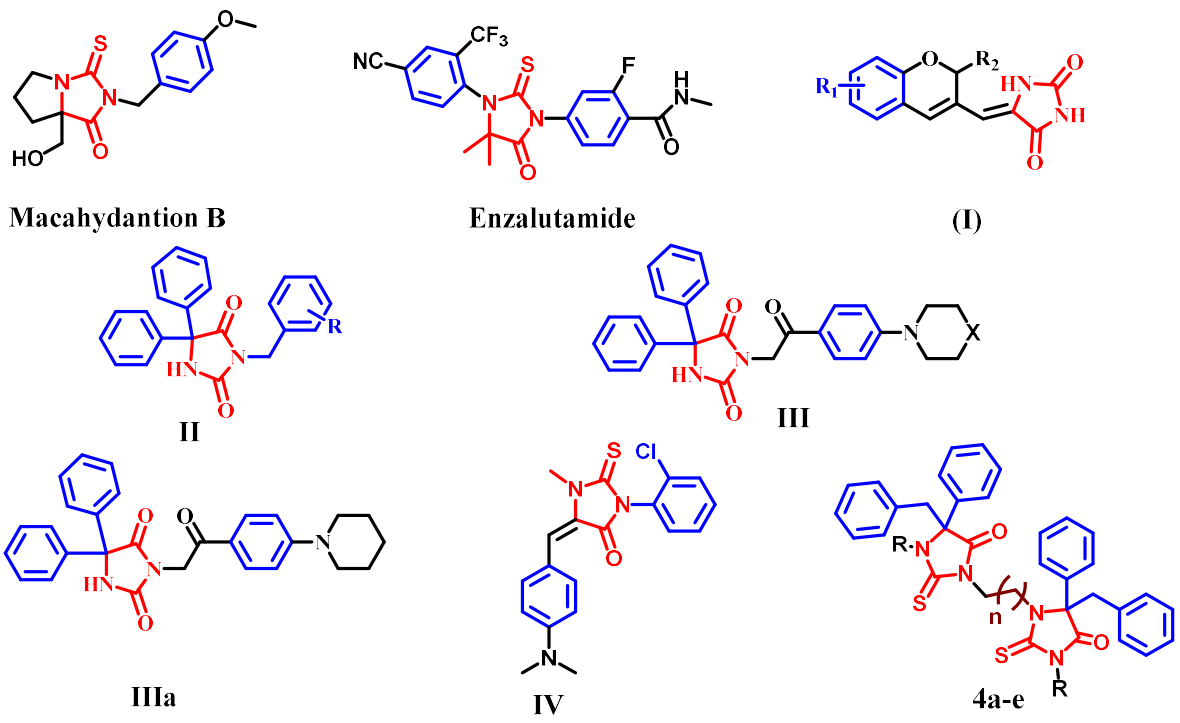


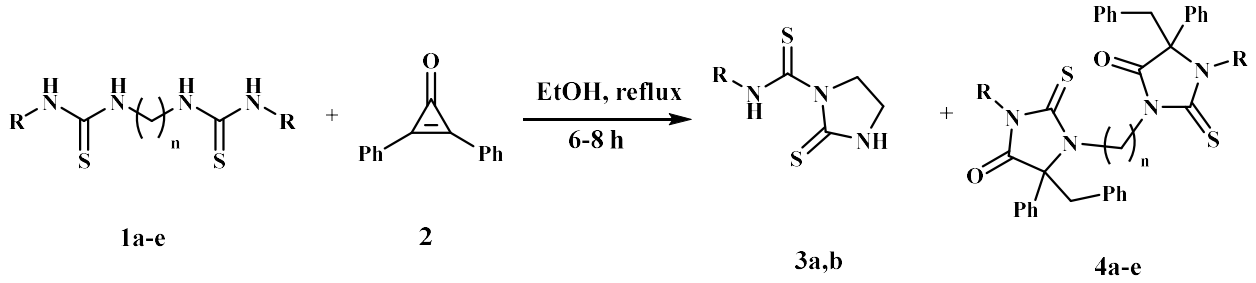
Fig. 1: Structure of hydantoin/thiohydantoin-based derivatives and targets **4a-e**

Based on the foregoing, we have developed a small set of thiohydantoin-based derivatives **4a-e** (**Fig. 1**) to be investigated as antiproliferative agents targeting EGFR-TK. In a cell viability assay, the newly developed compounds will be evaluated for their safety profile against normal human cancer cell lines. Further, compounds **4a-e** will be tested against a panel of four human cancer cell lines to determine their IC_{50} values. The most potent compounds will be tested further as EGFR inhibitors. Finally, a molecular docking research will be performed to investigate how these compounds interact with the EGFR active site.

2. Results and Discussion:

2.1. Chemistry

Scheme 1 depicts the synthetic method for the synthesis of the target compounds **4a-e**. We began our reaction by investigating several aspects. First, different bis[organylthiourea] derivatives **1a-e**, were screened (**Scheme 1**). Second, the reaction was carried out in different solvents such as absolute ethanol, acetonitrile, toluene, cyclohexane and dimethylformamide (DMF). However, the yields of **4a-e** decrease when using toluene, cyclohexane, acetonitrile and DMF. Finally, by adding excess of the reaction contents, namely diphenylcyclopropenone (**2**) or bithioureas **1a-e**, led to a significant decrease in the yields. There are no reported similar reactions of **2** with the *N*,*N*''-(1,-alkanediyl)bis(*N*'-organyl thiourea) derivatives **1a-e**. As a result, the reactions of these groups urge further exploration of the reactivity of **1a-e** toward **2**.



Scheme 1. Synthesis of compounds **4a-e**

Compound	R	n	Yield of 4 (%)
3a, 4a	Allyl	2	68%
3b, 4b	Ph	2	65%
4c	Allyl	3	63%
4d	Et	3	66%
4e	Ph	3	60%

This study was begun by stirring under reflux a solution of *N*,*N*''-(1,*ω*-alkanediyl)bis(*N*′-organyl thiourea) derivatives **1a,b** [17, 18] and 2,3-diphenylcyclopropanone (**2**) in absolute ethanol for 6-8 h. The reaction mixture was concentrated and subjected under chromatographic plates to give 3-substituted-1-(2-(3-substituted-4-benzyl-5-oxo-4-phenyl-2-thioxoimidazolidin-1-yl)ethyl)-5-benzyl-5-phenyl-2-thioxo-

imidazolidin-4-ones **4a-e** as the major products (68-60%) and *N*-phenyl-2-thioxo-imidazolidine-1-cashothioamide **3a** and **3b** (6-4%) as a minor products (the structure of **3b** was confirmed by X-ray crystallographic analysis, see SI). Compounds **3a** [27] and **3b** [28] are well-known compounds. Different spectroscopic methods of analysis were used to confirm the structures of compounds **4a-e**. The structures of (thioimidazolidinyl)ethyl-2-thioxoimidazolidinone derivatives **4a-e** showed the stretching frequency range between ν = 2980 and 2960 cm^{-1} due to aliphatic-CH_2, 1680-1664 cm^{-1} for amide-CO, and intense band in the range of 1378 – 1351 and 1028 - 976cm^{-1} assigned to strongly coupled between C=S and C-N vibrations. The ^{1}H NMR spectrum of **4a** (in $CDCl_3$) as an example showed sharp singlet at δ_H = 3.62 ppm with integration equal to four protons due to $(CH_2)_2$ group; three multiplets were appeared; one at δ_H = 4.12, 4.01 with four protons for two allyl-CH_2N, and another two multiplets at δ_H 5.12-5.09 due to allyl-CH_2= and at δ_H 5.86-5.91 for allyl-CH=, and finally doublet of doublet signals at δ_H = 4.75 and 4.87 ppm with (J = 3.62 Hz) for diastereotopic benzyl-CH_2 group. The ^{13}C NMR spectra of compounds **4a-e** showed carbon signals at δ_C = 51.22 and 53.16 ppm which was assigned to $(CH_2)_2$. Two carbon signals; one at δ_C = 56.63 and other at δ_C = 56.66 ppm assigned to CH_2Ph. Carbon signals were also appeared at δ_C = 90.17 (chiral-C, C-4), 175.12 (amide C=O) and 181.12 ppm (cyclic C=S). For **4a**, mass spectroscopy revealed an ion peak at *m/z* = 588 represents the fragment formed by release of the corresponding two allyl groups from the molecular ion.

To prove the structure of compounds **4a-e** unambiguously, single crystals of **4a** was obtained. The X-ray crystallographic analysis confirmed the molecular structure of compounds **4a-e** and presence of two stereogenic centers and the molecule is *meso*. The structure of 3-allyl-1-(2-(3-allyl-4-benzyl-5-oxo-4-phenyl-2-thioxo-imidazolidin-1-yl)ethyl)-5-benzyl-5-phenyl-2-thioxoimidazolidin-4-one (**4a**) was determined by X-ray analysis (**Fig. 2**, Table S1 in supplementary data). The imidazolidine rings have only a slightly non-planar conformation (mean deviation from the L.S.-plane N3-C4-N5-C6-C7 and N24-C25-N26-C27-C28 are 0.018 Å and 0.008 Å, respectively) and are twisted by 26° (angle between the two L.S.-planes).

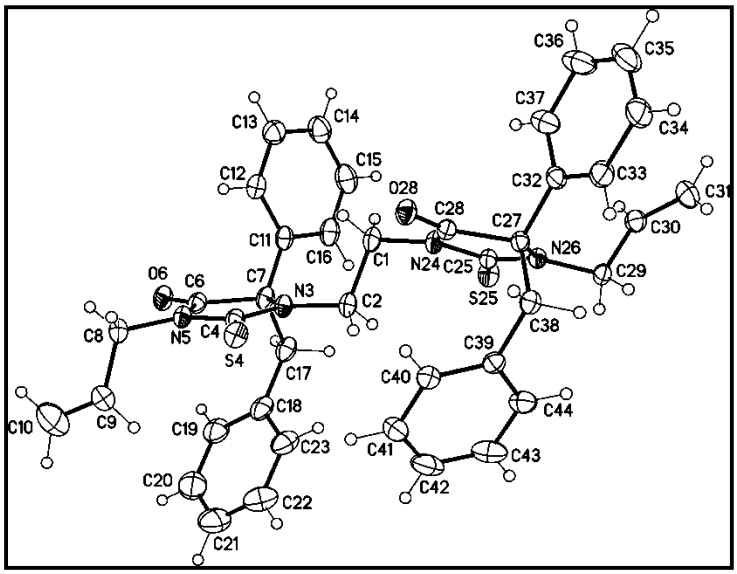
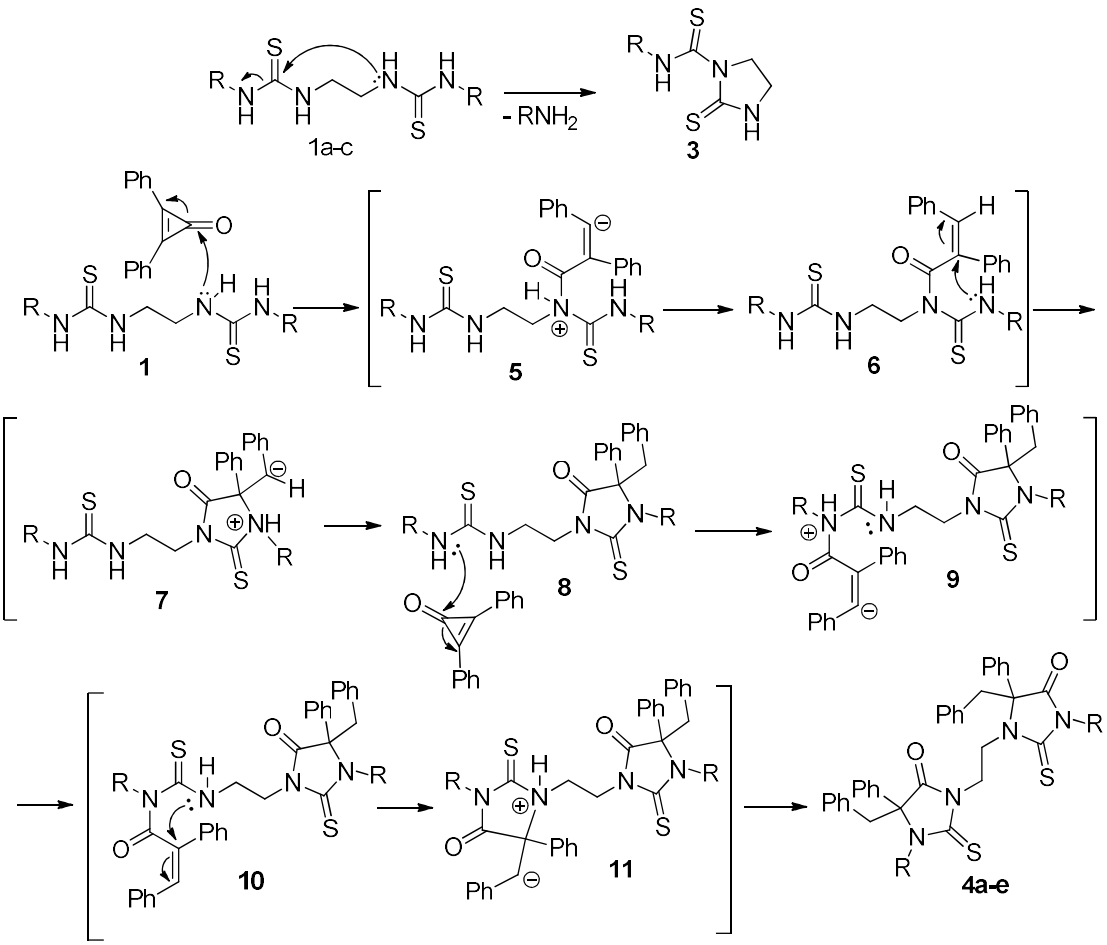


Fig. 2: X-ray structure analysis of 3-allyl-1-(2-(3-allyl-4-benzyl-5-oxo-4-phenyl-2-thioxo-imidazolidin-1-yl)ethyl)-5-benzyl-5-phenyl-2-thioxoimidazolidin-4-one (**4a**) (displacement parameters are drawn at 30% probability level)

Scheme 2 cites a possible reaction mechanism based on the condition of our reaction. Firstly, the formation of compounds **3a** and **3b** was described as due to the internal cyclization by the nucleophilic addition of the nitrogen lone pair of one thiourea to the electrophilic-C of the C=S in the second thiourea group. The latter process accompanied with extrusion of one molecule of amine. Secondly, formation of compounds **4a-e** was explained as due to the addition of **1a-e** on the carbonyl group in **2** to form intermediate Zwitter-ion **6**. Subsequently, nucleophilic addition of thioamide-NH to conjugated C=C afforded the formation of intermediate **7,** which would be neutralized into **8**. On repeating the previous steps of the reaction with the second thiourea group in **1a-c** with another molecule of **2**, intermediates **9** to **11** would be formed and consequently compounds **4a-e** would be obtained (**Scheme 2**).



Scheme 2. The plausible mechanism for the formation of compounds **3a**, **3b**, and **4a-e**.

2.2. Biology

2.2.1. Cell Viability assay

The human mammary gland epithelial (MCF-10A) cell line was used to test the viability of novel compounds **4a-e** [19, 20]. MCF-10A cells were treated for four days with **4a-e** before being tested for viability using the MTT assay. According to **Table 1**, none of the compounds tested were cytotoxic, and cell viability was greater than 87% for the compounds tested at 50 μM.

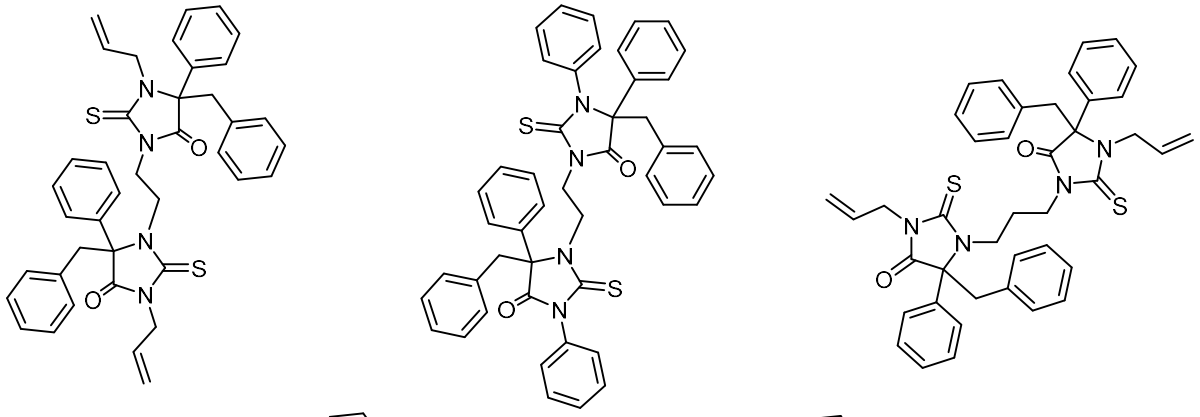
2.2.2. Antiproliferative assay

Compounds **4a-e** were tested for antiproliferative activity against four human cancer cell lines using the MTT assay [21-24] and doxorubicin as the reference drug: Panc-1 (pancreas cancer cell line), MCF-7 (breast cancer

cell line), HT-29 (colon cancer cell line), and A-549 (epithelial cancer cell line). **Table 1** displays the median inhibitory concentration (IC_{50}).

In general, the tested compounds **4a-e** demonstrated promising antiproliferative activity against the four cancer cell lines, with GI_{50} ranging from 1.20 μM to 4.00 μM in comparison to the reference doxorubicin (GI_{50} = 1.10). Compounds **4c-e**, n = 3, were more potent than compounds **4a** and **4b**, where the linker was only two carbon atoms (n = 2), indicating the importance of linker length in these compounds' antiproliferative effect. Compound **4c** (R = allyl, n = 3) was the most potent, with an average GI_{50} value of 1.20 μM, which was equivalent to the reference doxorubicin's GI_{50} value of 1.10 μM. In terms of activity against the four cancer cell lines, compound **4c** is almost as effective as doxorubicin. Replacement of the allyl group in compound **4c** with the phenyl group in compound **4e** (R = phenyl, n = 3) resulted in a slight decrease in antiproliferative activity of compound **4e**, with a GI_{50} of 1.50 μM compared to 1.20 μM for **4c**. Furthermore, replacing the allyl group in **4c** with ethyl group in **4d** (R = ethyl, n = 3) resulted in a significant decrease in antiproliferative effect, with compound **4d** GI_{50} equal to 1.70 μM being 1.4-fold less potent than **4c**. These results illuminated the significance of the substitution pattern at the imidazolidine moiety's position 3, where allyl group exhibit the highest activity, followed by phenyl group, and ethyl group exhibit the lowest action. Finally, compounds **4a** (R = allyl, n = 2) and **4b** (R = phenyl, n = 2) had lowest activity with GI_{50} values of 4.00 μM and 2.40 μM, respectively being 3-folds and 1.5-folds less potent than **4c** (R = allyl, n = 3) and **4e** (R = phenyl, n = 3), respectively.

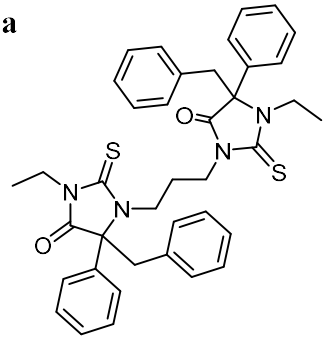
Table 1: IC_{50} of compounds **4a-e** and Doxorubicin



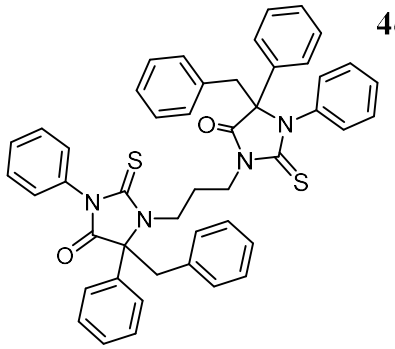
4a

4b

4c



4d



4e

Compd.	Cell viability %	Antiproliferative activity IC_{50} ± SEM (nM)				
		A-549	MCF-7	Panc-1	HT-29	Average
4a	**89**	3.90±0.30	3.70±0.30	4.20±0.30	4.20±0.30	4.00
4b	**86**	2.40±0.20	2.10±0.20	2.60±0.20	2.60±0.20	2.40
4c	**91**	1.30±0.10	1.00±0.10	1.40±0.10	1.20±0.10	1.20
4d	**90**	1.60±0.10	1.50±0.10	1.80±0.10	1.80±0.10	1.70
4e	**85**	1.40±0.10	1.20±0.08	1.60±0.10	1.60±0.10	1.50
Doxorubicin	-	1.20 ± 0.10	0.90± 0.10	1.40 ± 0.10	1.00 ± 0.10	1.10

2.2.3. EGFR inhibitory assay

The EGFR-TK assay [25, 26] was used to evaluate the EGFR inhibitory potency of compounds **4b-e**; the results are displayed in **Table 2**. The tested compounds showed EGFR inhibitory activity with IC_{50} values ranging from

90 nM to 178 nM in comparison to the reference erlotinib which had an IC_{50} value of 70 nM. The tested compounds in every instance were less effective than erlotinib. The outcomes of this test were consistent with those of a cancer cell-based assay, in which compound **4c** (R = allyl, n = 3), the most potent antiproliferative, had the highest inhibitory effect on EGFR with an IC_{50} value of 90 nM, as opposed to erlotinib's IC_{50} value of 70 nM. The second and third-most active compounds were **4e** (R = phenyl, n = 3) and **4d** (R = ethyl, n = 3) and with IC_{50} values of 107 nM and 128 nM. Finally, compound **4b** (R = phenyl, n = 2) was the least effective EGFR inhibitor, with an IC_{50} value of 178 nM. These findings imply that the compounds tested had a significant antiproliferative effect as well as the ability to act as an EGFR inhibitor.

Compound	EGFR $IC_{50} \pm$ SEM (nM)
4b	178 ± 12
4c	90 ± 7
4d	128 ± 10
4e	107 ± 8
Erlotinib	70 ± 5

2.3. Molecular Docking Simulations

As discussed before in results and discussions, how effective are the 2-thioxo-4-imidazolidin-4-ones as EGFR inhibitors, we decided to explore their possible interaction modes within active sites of EGFR. Molecular docking simulations of these compounds within EGFR active site revealed their good interaction profile as summarized in **Table 3**. Compound **4c** showed the highest docking score (S; kcal/mol) within five test compounds.

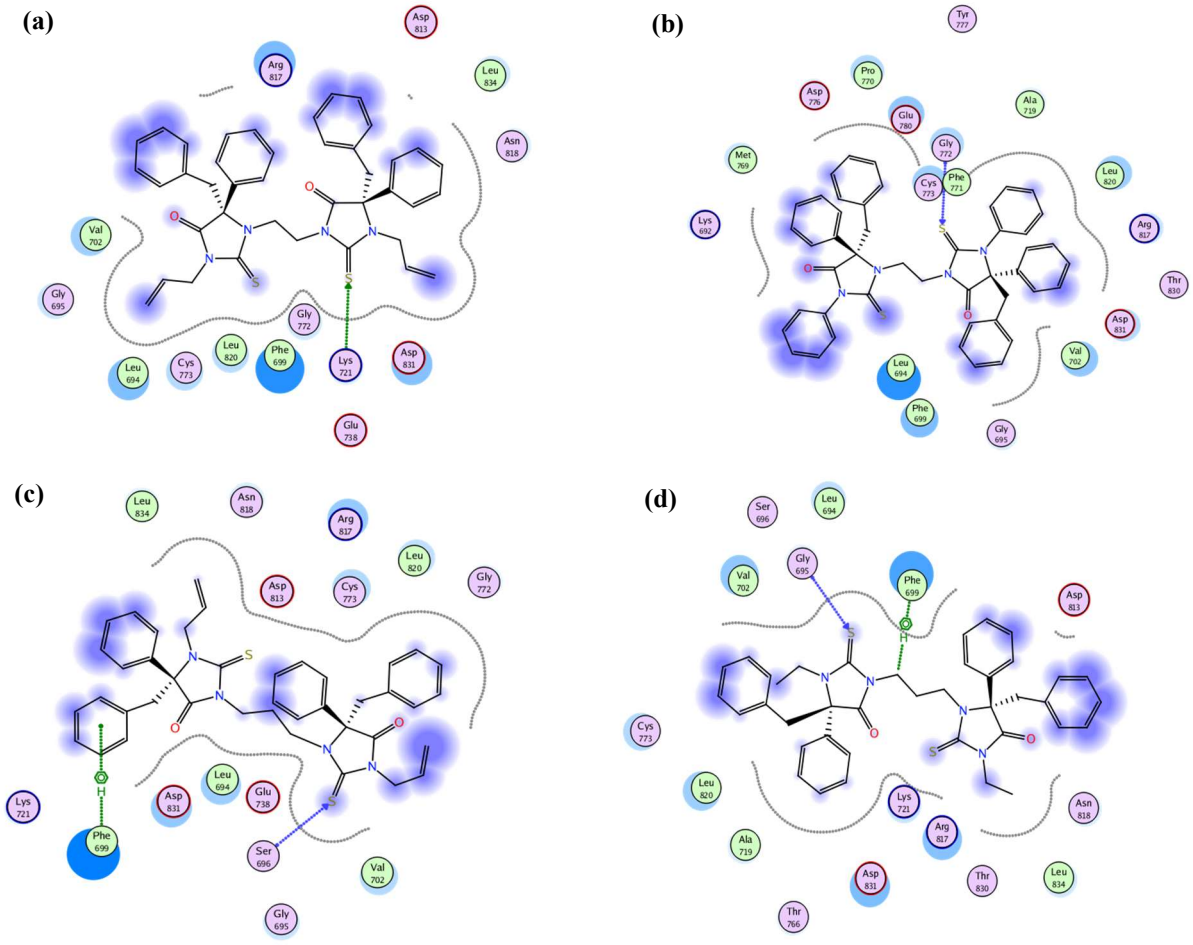
Visual inspections of binding interactions of best docking pose of each of the five test compounds and co-crystallized ligand (Erlotinib), showed stabilization of their molecules inside cavity of active site with number of H-bonds and pi-H hydrophobic interactions with various amino acid residues lining active site, as shown

in **Figs. 3** and **4**. Compound **4c** has two hydrogen bonds with Ser 696 and Phe 699 where Erlotinib form one hydrogen bond with Ser 696.

Table 3. Binding Interactions of **4a-e** and Erlotinib within EGFR (PDB ID: 1M17) active sites.

	4a	4b	4c	4d	4e	Erlotinib
EGFR (PDB ID): 1M17						
S (kcal/mol)	-6.44	-6.56	-6.88	-6.78	-6.82	-6.27
RMSD (Å)	1.75	1.76	1.93	1.64	1.42	1.46
Amino acids residues binding interactions & their bond length (Å)	Lys 721 (3.96)[a]	Gly 772 (4.00)[a]	Ser 696 (3.99)[a] Phe 699(3.86)[b]	Gly 695 (3.95)[c] Phe 699 (4.12)[b]	Lys 721 (3.98) Phe 699 (3.93)[b] Leu 694 (4.21)[b]	Ser 696 (3.46)[a]

[a] H-acceptor; [b] pi-H; [c] H-donor



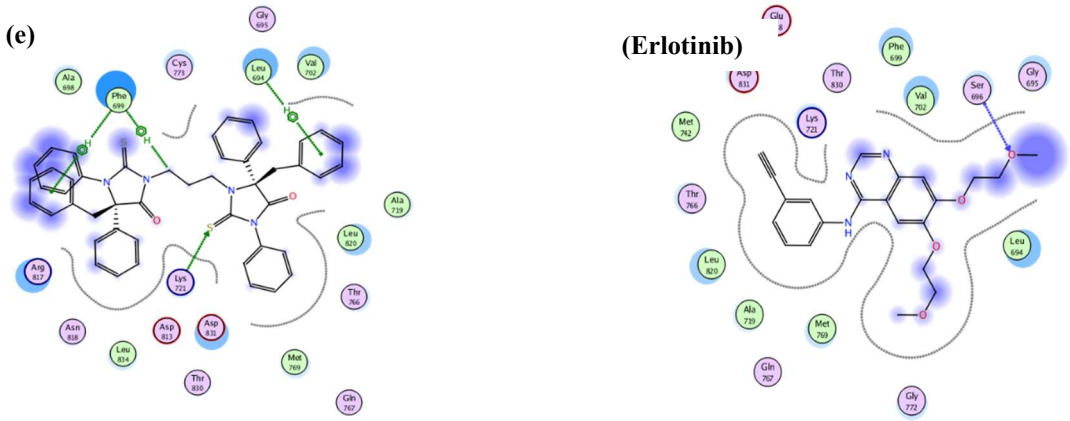


Fig. 3. 2D Interaction diagram of **4a-e** & **Erlotinib** within EGFR (PDB ID: 1M17) active site showing H-bonding (green and blue arrows), pi-H (green dotted-line), and proximity contour around each molecule (grey dotted-line)

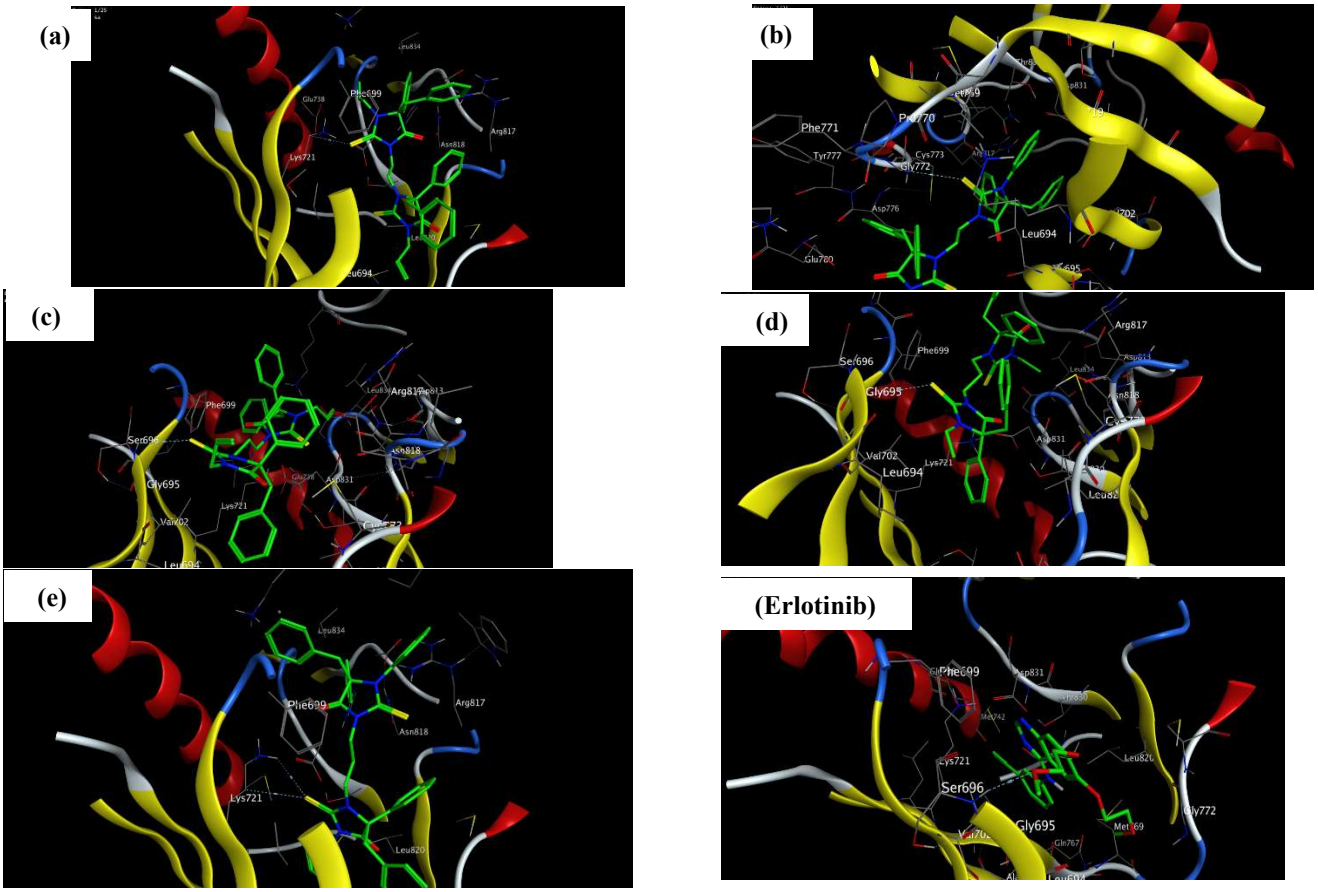


Fig. 4. 3D Interaction diagram of **4a-e** & Erlotinib within EGFR (PDB ID: 1M17)

3. Conclusions

In conclusion, we have successfully developed a new strategy for the preparation of (2-(3-substituted-4-benzyl-5-oxo-4-phenyl-2-thioxoimidazolidin-1-yl)ethyl)-5-benzyl-5-phenyl-2-thi-oxoimidazolidin-4-ones through nucleophilic addition of N,N''-(1,ω-alkanediyl)bis(N'-organyl thiourea) derivatives and ring opening of 2,3-diphenylcyclopropanone. One the basis of the expected biological activity of the formed imidazole thione moiety the antiproliferative activity of the obtained products was also investigated. In comparison to erlotinib, which served as a control and had an IC_{50} value of 70 nM, the compounds tested showed EGFR inhibitory activity with IC_{50} values ranging from 90 nM to 178 nM. The most effective antiproliferative agent was discovered to be compound **4c**. When compared to erlotinib, which has an IC_{50} value of 70 nM, it had the most potent inhibitory effect on EGFR, with an IC_{50} value of 90 nM. Compounds **4e** and **4d** were the second and third most active compounds, with IC_{50} values of 107 nM and 128 nM, respectively. The compounds studied were both EGFR inhibitors and had a significant antiproliferative effect, according to the findings. The new compounds reported here are currently undergoing structural modifications in order to synthesize a new series of compounds that will be subjected to additional *in vitro* and *in vivo* assays in the hopes of obtaining a lead compound for drug design.

4. Experimental:

4.1.Chemistry

General details: See Appendix A (Supplementary File)

N,*N*''-(1,*ω*-Alkanediyl)bis(*N*'-organyl thiourea) derivatives **1a-e** were prepared according to literature methods [17, 18]

4.1.1. Syntheses of imidazolidinethione and bis imidazolidinethione derivatives (3a, 3b, and 4a-e)

General procedure:

A solution of **1a-e** (1.0 mmol) in absolute ethanol (20 ml), a solution of **2** (1.0 mmol) in absolute ethanol (20 ml) was added dropwise with stirring. The mixture was stirred for 30 min, then the mixture was refluxed for 6-8 h (the reaction was monitored by TLC analyses). The reaction mixture was concentrated, and the residue was subjected to chromatographic plates using toluene-ethyl acetate (5:2) as eluent. The fastest migration zone contains the imidazolidine thione derivatives **3a,b** (4-6%), the slowest migrating zone containing bis-imidazolidine thione derivatives **4a-e** (68-60%), the products obtained were recrystallized from the stated solvents.

N-Allyl-2-thioxoimidazolidine-1-carbothioamide (**3a**). 4%, mp 123–125 (lit. 126–128) °C [27].

N-Phenyl-2-thioximidazolidine-1-carbothioamide (**3b**). 6%, mp 176–177 (lit. 176–177) °C [28].

4.1.1.1. *(R)-3-Allyl-1-[2-(S)-3-allyl-4-benzyl-5-oxo-4-phenyl-2-thioxoimidazolidin-1-yl)ethyl)-5-benzyl-5-phenyl-2-thioxoimidazolidin-4-one* (**4a**)

Colorless crystals (EtOH); m.p 268: 270 ºC ; yield:456.2 mg (68 %); IR: 3090-3081 (Ar-CH), 2966 (ali-CH_2) 1686 (C=O), 1361 cm^{-1} (C=S and C-N); ^{1}H NMR ($CDCl_3$): δ_H = 3.37-3.62 (m, 4H, -CH_2), 4.02-4.12 (m, 4H, allyl-CH_2N), 4.75-4.77 (d, d, *J* = 3.62 Hz, 4H, benzyl-CH_2), 5.09-5.12 (m, 4H, allyl-CH_2=), 5.86-5.91 (m, 2H, allyl-CH=), 7.03-7.11 (m, 8H, Ar-H), 7.21-7.28 (m, 8H, Ar-H), 7.38-7.41, 7.48, 7.53 ppm (m, 4H, Ar-H); ^{13}C NMR ($CDCl_3$): δ_C = 51.23, 53.16 (CH_2), 54.48 (allyl-CH_2), 56.63, 56.66 (benzyl-CH_2), 90.17

(C-4, C), 116.53 (allyl-CH_2=), 126.95, 127.12, 127.16,128.12, 128..39, 128.84, 129.12, 129.36 (Ar-CH), 131.12, 132.44, 133.71 (Ar-C), 135.45 (allyl-CH=), 175.12 (C=O), 181.12 ppm (C=S); MS (70 eV, %): *m/z* = 670 (M^+, 41), 588 (18), 335 (87), 92 (100), 41 (76). Anal. Calcd. For $C_{40}H_{38}N_4O_2S_2$ (670.89): C, 71.61; H, 5.71; N, 8.35; S, 9.56. Found C,71.73; H, 5.80; N,8,41; S, 9.59.

4.1.1.2. ***(S)-5-Benzyl-3-[2-(R)-5-benzyl-4-oxo-3,5-diphenyl-2-thioxoimidazolidin-1-yl)ethyl)-1,5-diphenyl-2-thioxoimidazolidin-4-one*** (**4b**)

Colorless crystals (CH_3CN); m.p 260: 262 ºC ; yield 482.9 mg (65 %); IR: 3100-3085 (Ar-CH), 2954-2942 (ali-CH_2) 1684 (C=O), 1358 cm^{-1} (C=S and C-N); ^{1}H NMR ($CDCl_3$): δ_H = 3.22 (s, 4H, -CH_2), 3.41-3.74 (s, 4H, benzyl-CH_2), 6.80-7.15 (m, 10H, Ar-H), 7.40-7.53 ppm (m, 10H, Ar-H); ^{13}C NMR ($CDCl_3$): δ_C = 41.98, 41.62 (benzyl-CH_2), 55.22 (CH_2),), 92.17 (C-5), 126.12, 126.81, 127.69, 127.79, 128.11, 128.18, 128.25, 128.37,128. 52, 129.30, 129.39 (Ar-CH), 135.76, 138.03, 141.71, 142.84 (Ar-C), 173.64 (C=O), 178.84 ppm (C=S); MS (70 eV, %): *m/z* = 742 (M^+, 67), 558 (46), 371 (100), 92 (82). Anal. Calcd. For $C_{46}H_{38}N_4O_2S_2$ (742.95): C, 74.36; H, 5.16; N, 7.54; S, 8.63. Found C,74.48; H, 5.22; N,7.46; S, 8.58.

4.1.1.3. ***(R)-3-Allyl-1-[3-(S)-3-allyl-4-benzyl-5-oxo-4-phenyl-2-thioxoimidazolidin-1-yl)propyl)-5-benzyl-5-phenyl-2-thioxoimidazolidin-4-one*** (**4c**)

Colorless crystals (CH_3OH); m.p 243: 245ºC ; yield: 431.4 mg (63 %); IR (KBr): υ = 3086-3078 (Ar-CH), 2978-2966 (ali-CH_2) 1681 (C=O), 1358 cm^{-1} (C=S and C-N); ^{1}H NMR ($CDCl_3$): δ_H = 1.76 (m, 2H, CH_2), 3.68 (t, 2H, CH_2), 4.21 (t, 2H, CH_2), 4.25-4.27 (m, 4H, allyl-CH_2N), 4.73, 4.85 (d,d, 4H, benzyl-CH_2), 5.11-514 (m, 4H, allyl-CH_2=), 5.89-5.92 (m, 2H, allyl-CH=), 7.09-7.11 (m, 8H, Ar-H), 7.31-7.56 ppm (m, 12H, Ar-H); ^{13}C NMR ($CDCl_3$) δ_C = 25.16 (CH_2), 49.62 (CH_2), 55.07 (allyl-CH_2), 50.06 (CH_2), 90.64 (C-4,), 116.74 (allyl-CH_2=), 125.9, 126.6, 127.6, 127.7,128.6, 129.2, 129.6 (Ar-CH), 131.76, 132.64, 133.84 (Ar-C), 135.83 (allyl-CH=), 174.93 (C=O), 181.28 ppm (C=S); MS (70 eV, %): *m/z* = 684 (M^+, 54), 642 (28), 352

(37), 358(18), 92 (36), 42 (38). Anal. Calcd. For $C_{41}H_{40}N_4O_2S_2$ (684.91): C, 71.90; H, 5.89; N, 8.18; S, 9.36. Found C,71.98; H, 5.94; N,8,09; S, 9.42.

4.1.1.4. ***(S)-5-Benzyl-3-[3-(R)-5-benzyl-3-ethyl-4-oxo-5-phenyl-2-thioxoimidazolidin-1-yl)propyl)-1-ethyl-5-phenyl-2-thioxoimidazolidin-4-one*** (**4d**)

Colorless crystals (CH_3CN); m.p 254: 256 ^oC ; yield: 436.18 mg (66 %); IR: 3072-3064 (Ar-CH), 2984-2964 (ali-CH_2) 1684 (C=O), 1364 cm^{-1} (C=S and C-N); ^1H NMR ($CDCl_3$): δ = 1.28 (t, 6H, $2CH_3$, J = 7.01), 1.69 (m, 2H, CH_2), 3.06, 3.38 (s, 2H, benzyl-CH_2), 3.42 (t, 2H, -CH_2), 3.51 (q, 2H, ethyl-CH_2 J =), 4.08 (q, 2H, ethyl-CH_2, J = 7.01), 4.12 (t, 2H, CH_2), 6.96-7.27 (m, 10 H, Ar-H), 7.28-7.54 ppm (m, 10H, Ar-CH); ^{13}C NMR ($CDCl_3$) δ_C = 12.64 (CH_3), 13.37 (CH_3), 41.22, 41.10 (benzyl-CH_2), 41.53 (chiral-C), 24.71 (CH_2), 41.32 (benzyl-CH_2), 45.76 (CH_2-ethyl), 46.63 (CH_2-ethyl), 47.93 (CH_2), 49.62 (CH_2), 126.32, 126.81, 127.76, 127.71, 128.80, 129.22, 129.63 (Ar-C), 135.53, 139.46, 141.12, (Ar-C), 177.41 (C=O), 178.55 ppm (C=S); MS (70 eV, %): m/z = 660 (M^+, 74), 631 (22), 602 (19), 337 (31), 309 (21), 92 (64), 29 (33). Anal. Calcd. For $C_{39}H_{40}N_4O_2S_2$ (660.84): C, 70.88; H, 6.10; N, 8.48; S, 9.70. Found C,70.76; H, 6.14; N,8.53; S, 9.67.

4.1.1.5. ***(S)-59-Benzyl-3-[3-(R)-5-benzyl-3-ethy]-4-oxo-3,5-diphenyl-2-thioxoimidazolidin-1-yl)propyl)-1,5-diphenyl-2-thioxoimidazolidin-4-one*** (**4e**)

Colorless crystals (CH_3CN), m. p. 242: 244^oC ; yield 454.18 mg (60 %); IR (KBr): υ = 3068-3063 (Ar-CH), 2988-2976 (ali-CH_2) 1677 (C=O), 1358 cm^{-1} (C=S and C-N); ^1H NMR ($CDCl_3$): δ_H = 1.71-1.74 (m, 2H, CH_2), 3.11, 3.46 (s, 2H, benzyl-CH_2), 3.48 (m, 2H, -CH_2), 4.11 (m, 2H, CH_2), 6.65-6.80 (m, 4H, Ar-H), 7.21-7.25 (m, 9H Ar-CH), 7.27-7.29 (m, 9H, Ar-CH), 7.40-7.43 ppm (m,8H, Ar-CH); ^{13}C NMR ($CDCl_3$) δ = 24.31 (CH_2), 47.91 (CH_2), 41.12, 40.48 (benzyl-CH_2), 90.63, 90.82 (chiral-C), 139.26, 136.51, 141.42, 140.54, 140.94 (Ar-C), 177.41 (C=O), 178.55 ppm (C=S); MS (70 eV, %): m/z = 756.2 (M^+, 46), 572 (38), 371 (26), 399 (29), 92 (61). Anal. Calcd. For $C_{47}H_{40}N_4O_2S_2$ (756.2): C, 74.57; H, 5.33; N, 7.40; S, 8.47. Found C,74.61; H, 5.28; N,7.46; S, 8.41.

4.2. Crystal Structure Determinations of 3b and 4a

The single-crystal X-ray diffraction studies were carried out on a Bruker D8 Venture diffractometer with Photon II detector at 173(2) K using Cu-Kα radiation (λ = 1.54178 Å). Dual space methods (SHELXT for **5a**) [G. M. Sheldrick, *Acta Crystallogr.* 2015, **A71**, 3-8] were used for structure solution and refinement was carried out using SHELXL-2014 (full-matrix least-squares on F^2) [G. M. Sheldrick, *Acta Crystallogr.* 2015, **C71**, 3-8]. Hydrogen atoms were refined using a riding model. Semi-empirical absorption corrections were applied.

3b: colourless crystals, $C_{10}H_{11}N_3S_2$, M_r = 237.34, crystal size 0.20 × 0.12 × 0.04 mm, monoclinic, space group $P2_1/c$ (No. 14), a = 7.4431(5) Å, b = 12.1308(8) Å, c = 12.2679(8) Å, β = 103.162(3)°, V = 1078.58(12) Å^3, Z = 4, ρ = 1.462 Mg/m^{-3}, μ(Cu-K$_\alpha$) = 4.22 mm^{-1}, F(000) = 496, T = 173 K, $2\theta_{max}$ = 144.6°, 10057 reflections, of which 2125 were independent (R_{int} = 0.035), 196 parameters, 615 restraints (see cif-file for details), R_1 = 0.047 (for 2034 I > 2σ(I)), wR_2 = 0.127 (all data), S = 1.09, largest diff. peak / hole = 0.59 / -0.30 e Å^{-3}. Disorder of the complete molecule (about a 2-fold axis), 90:10 (determined at the stage of the isotropic refinement and then fixed), minor disordered part refined isotropically (see cif-file for details).

4a: colourless crystals, $C_{40}H_{38}N_4O_2S_2$, M_r = 670.86, crystal size 0.21 × 0.18 × 0.03 mm, monoclinic, space group $P2_1/n$ (No. 14), a = 11.7727(3) Å, b = 13.6117(3) Å, c = 22.3014(5) Å, β = 92.596(1)°, V = 3570.05(14) Å^3, Z = 4, ρ = 1.248 Mg/m^{-3}, μ(Cu-K$_\alpha$) = 1.67 mm^{-1}, F(000) = 1416, T = 173 K, $2\theta_{max}$ = 144.8°, 37307 reflections, of which 7054 were independent (R_{int} = 0.029), 433 parameters, 387 restraints (see cif-file for details) R_1 = 0.057 (for 6383 I > 2σ(I)), wR_2 = 0.160 (all data), S = 1.04, largest diff. peak / hole = 0.78 / -0.28 e Å^{-3}.

CCDC 2235115 (**3b**), and 2235116 (**4a**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Acknowledgements

We gratefully acknowledge the support of the Karlsruhe Institute of Technology's KIT-Publication Fund. The National Science Foundation of the United States aided in the purchase of the NMR spectrometer at Florida Institute of Technology (CHE 03 42251).

Conflict interest statement: The authors declare no conflict of interest.

Funding: No Funds

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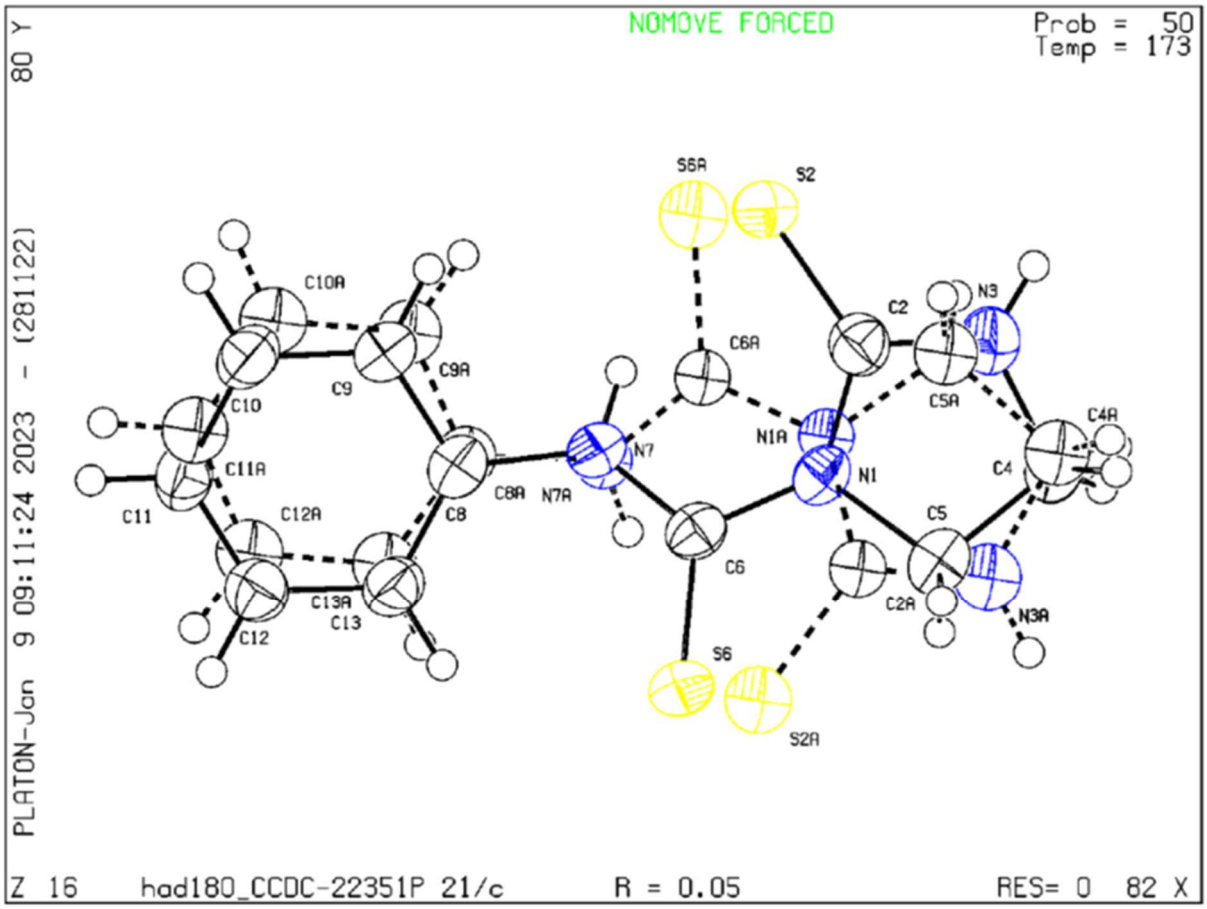

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