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Farmaceutická fakulta v Hradci Králové

Katedra farmaceutické chemie a kontroly léčiv

Design and synthesis of BCA2 inhibitors

(Design a syntéza BCA2 inhibitorů)

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Hradec Králové, 2010

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* This thesis was processed as part of Erasmus program at the Welsh School of Pharmacy at the Cardiff University

Declaration

"This thesis is the result of my own investigation, except where otherwise stated. Other sources are acknowledged by explicit references."

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V Hradci Králové …… ……..

podpis

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Abstract

BCA 2 (Breast Cancer Associated Protein2) is a novel monomeric RING (Really Interesting New Gene 2) – type ubiquitin E3 ligase. It was found to be overexpressed in 56 % of invasive breast cancers and its expression is correlated with a positive estrogen receptor status.

The RING-type family of proteins possesses ubiquitin ligase activity and involves in protein degradation. Ubiquitylation is used to target proteins of different biological processes including proteosomal degradation or endocytosis. Polyubiquitination of target protein substrates is carried out by three classes of ubiquitin ligase enzymes, of which the diverse E3 ligase family catalyse the final step of ubiquitin transfer to specific lysyl residues of target proteins prior to proteosomal destruction. The RING-type proteins can be defined as unique linear series of conserved cysteine and histidin residues and binds two zinc atoms in a cross-brace arrangement.

Studies of zinc-ejecting compounds have led to the identification of disulfiram, which has been used for alcohol aversion therapy for alcohol aversion therapy as an alcohol dehydrogenase inhibitor.

In this thesis I describe the synthesis of three series of novel zinc-affinic compounds, in order to optimise selectivity of BCA2 inhibitors which could lead to the identification of novel more effective antitumor agents compared to disulfiram.

Abstrakt

BCA2(Protein 2 spojený s rakovinou prsu) je nová monomerní E3 ligáza typu RING (Opravdu zajímavý gen 2). Byla zjištěna zvýšená exprese u 56% případů invazivní rakoviny prsu a tato exprese koreluje s pozitivním stavem estrogenového receptoru.

Rodina proteinů typu RING má ubichitin ligázovou aktivitu a zapojuje se do degradace proteinů. Ubichitinace slouží k označení proteinů v různých biologických procesech zahrnujících proteozomální degradaci nebo endocytózu. Polyubichitinace cílových proteinů je provedena třemi skupinami ubichitin ligázových enzymů, z nichž rozmanitá skupina E3 ligáz katalyzuje poslední stupeň přenosu ubichitinu ke specifickým lysylovým zbytkům cílových proteinů za účelem jejich destrukce. Proteiny typu RING mohou být definovány jako unikátní lineární série složená z cysteinových a histidinových zbytků vázajících dva atomy zinku ve zkříženém uspořádání.

Studie látek schopných vytěsňovat zinek vedly k identifikaci disulfiramu, který je používán v terapii alkoholismu jako inhibitor alkohol dehydrogenázy.

V této práci popisuji syntézu tří sérií nových látek s afinitou k zinku za účelem optimalizace selektivity BCA2 inhibitorů, což by mohlo vést k identifikaci nových a účinnějších protirakovinných látek ve srovnání s disulfiramem.

Content

List of figures and reaction schemes

Figures

Schemes

Abbreviations

ALDH – Aldehyde dehydrogenase

APC – Anaphase- promoting complex

BCA 2 - Breast Cancer Associated Protein 2

Br - Brom

C- Carbon

$CDCl_3$- Denatured chloroform

Cys - Cystein

D - Dublet

Ub – Ubiquitin

DCM- Dichloromethane

DMAP - 4-Dimethylaminopyridine

DMF - Dimethylformamide

DMSO – Dimethyl Sulfoxide

EA – Ethyl Acetate

EDCI - 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide

ER- Estrogen Receptor

EtOH – Ethanol

Gly - Glycine

H - Hydrogen

HCl – Hydrochloric Acid

Hx - Hexane

J - J-coupling

KOH – Potassium Hydroxide

M - Multiplet

N – Nitrogen

1N - 1 Molar solution

NaOH – Sodium Hydroxide

NMR – Nuclear Magnetic Spectra

OH – Hydroxyl Group

O – Oxygen

RING – Really Interesting New Gene

R.T – Room Temperature

S- Singlet

S_8 – Sulfur

SCF - Skp1-Culin-F-box protein

T - Triplet

TLC – Thin Layer Chromatography

TEA – Triethylamine

THF - Tetrahydrofuran

UPP- Ubiquitin Proteasome Pathway

Q - Quartet

Zn - Zinc

Chapter 1

Introduction

1.1 Breast Cancer

Breast cancer is the most common malignancy in women, one in four new cancers diagnosed worldwide each year is a cancer of the female breast and it is responsible for about 410,000 deaths in 2002 (1). Breast cancer risk is related to age; with 81 % of cases occurring in woman aged 50 years and over. The highest number of cases of breast cancer diagnosed is in the 50-69 age group (fig1.1) (2).

Figure 1.1: Numbers of new cases and age specific incidence rates, by sex, breast cancer, UK 2006

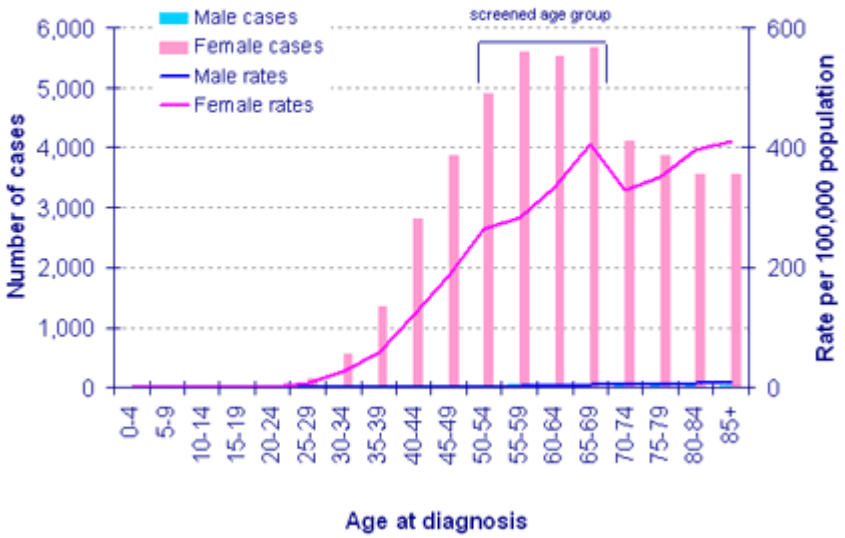


Fig.1.1

Other risk factors for breast cancer include reproductive factors, lifestyle factors, hormonal status and genetics. From reproductive factors breast cancer risk increases with decreasing age at menarche, increasing age at first pregnancy and low parity. Starting menarche at age 11 or earlier is considered as risk factor. Each birth reduces the relative risk of breast cancer by an average 7%. The reduction in risk per birth is greater for births at young age. A number of lifestyle factors have been linked to breast cancer. For example alcohol has been suggested as a risk factor in most studies. Some cohort studies have shown about a 30% increased risk of breast cancer among drinkers. Obesity is associated with an increased risk of breast cancer in postmenopausal women; the effect of large weight gains after 18 is 2% increasing risk per unit BMI. An increase in risk of breast cancer has been suggested in women with exposure to exogenous hormones, such as oral contraceptive use

or hormone replacement therapy. Also women with a family history of breast cancer are at increased risk of developing cancer (1). Woman with germ-line BRCA1/2 mutations are at significantly increased risk (3). The developing breast cancer for BRCA1/2 mutation carriers is 80-85% (1).

The optimal method for breast surveillance in woman is mammographic screening. Screening of postmenopausal women has been shown to reduce mortality, but its value in screening premenopausal women remains debatable. In the USA, mammography is recommended either annually; by contrast, most European countries recommend a screening interval of 2 years or longer (3).

Over recent years, there has been visible progress in treatment breast cancer. Pharmacological manipulation has been replacing ablative procedures. Endocrine therapy was essentially ablative to remove sources of estrogen by either surgery or radiotherapy. On the other hand, endocrine therapy is more useful for ER-positive tumors, which metastasise more frequently to soft tissue sites, bone and pleura. Chemotherapy is generally considered in cases of rapidly progressing disease and in potentially life-threatening situations, as well as when the disease is deemed hormone insensitive (f.e. ER negative tumor or tumor, which has developed de novo hormone resistance) (4).

Breast cancer endocrine therapies can be classified into three major types of therapeutic intervention, agents that directly target estrogen receptors, estrogen deprivation through aromatise inhibition or ovarian ablation, and sex steroid therapies (4).

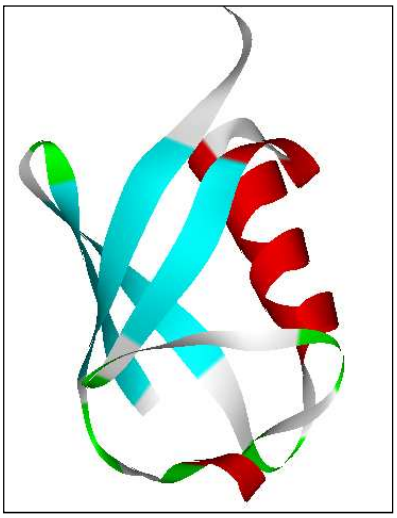
Tamoxifen is a competitive inhibitor of estrogen binding to the estrogen receptor. Tamoxifen has been described as the most important drug to be developed in the history of breast cancer. Its proven efficacy, combined with its good tolerability profile, makes tamoxifen the endocrine therapy of choice for all stages of breast cancer in women with ER-positive disease.

In addition the side effects of each approach are very distinct, with very important consequences for women´s health when considering long-term therapy (4). There is clearly a need for new chemical entities to treat breast cancer against new molecular targets.

In 2006, Burger et al. reported the discovery of a novel ring-type ligase BCA2. The protein has an autoubiquitination activity and it has been shown to be highly expressed in 56% of 945 invasive breast cancer cases. BCA2 might provide an alternative target for the treatment of hormone-refractory breast tumors (5).

Because BCA2 is involved in ubiquitination and protein degradation, the next sections will give an overview on the ubiquitination pathway and about the RING finger proteins and their role in ubiquitination.

1.2 Ubiquitin- mediated degradation



Nearly all proteins in mammalian cells are being degraded and replaced by de novo synthesis. The eukaryotic ubiquitin-proteasome system plays main role in protein homeostasis. Nearly 80% of cellular proteins are degraded by the proteasome in the cytoplasm and nucleus after being tagged with Ub (6). Ubiquitin is a 76-amino-acid globular protein (7) (fig 1.2).

Fig1.2. Topographical depiction of ubiquitin

Ubiquitylation is used to target proteins of different biological processes including proteasomal degradation or endocytosis. It has recently become clear that protein ubiquitylation also plays important roles in a large number of other biological processes, including DNA repair, transcription, translation, signal transduction, organelle assembly, protein trafficking, and virus budding (8).

Ubiquitin is covalently attached to other proteins through an iso-peptide bond between its carboxy-terminal glycine and the ε-amino group of lysines in the target protein. This can

be followed by further additions of ubiquitin to specific lysine residues within the linked ubiquitin itself, to generate a poly-Ub chain (9). Three enzymatic components are required to link chains of ubiquitin onto proteins that are destined for degradation. E1 (Ub-activating enzyme) and E2s (Ub-carrier or conjugating proteins) prepare ubiquitin for conjugation, but the key enzyme in the process is the E3 (Ub-protein ligase), because it recognizes a specific protein substrate and catalyzes the transfer of activated ubiquitin to it (10).

In mammalian organisms has been found only one type of E1, this abundant 110-kD enzyme uses ATP to generate an Ub thiolester, a highly reactive form of ubiquitin. The E2s generally are small proteins that share a conserved 16-kD core that contains the cysteine that forms a thiolester linkage with the activated ubiquitin. The large number of E2s helps to generate the specificity of the ubiquitination system, because specific E2s function in the degradation of various types of substrates, and they can conjugate with various E3s (10).

The ubiquitin-proteasome pathway is displayed in Fig.1.2. The C-terminal Gly residue of ubiquitin is activated in an ATP-requiring step by E1 (Step 1). This step consists of an intermediate formation of ubiquitin adenylate, with the release of PP_i, followed by the binding of ub to a Cys residue of E1 in a thiolester linkage, with the release of AMP. Activated ubiquitin is next transferred to an active site Cys residue of E2 (Step 2). Ubiquitin is linked by its C-terminus in an amide isopeptide linkage to ε-amino group of the substrate protein's Lys residues by E3 (Step 3). Proteins ligated to polyubiquitin chains are usually degraded by the 26S proteasome complex that requires ATP hydrolysis for its action (Step 4). The latter two products are converted to free and reusable ubiquitin by the action of ubiquitin-C-terminal hydrolases or isopeptidases (Steps 5 and 6). Some isopeptidases may also disassemble certain ubiquitin-protein conjugates (Step 7) and thus prevent their proteolysis by the 26S proteasome. Short peptides formed by this processes can be further degraded to free amino acids by cytosolic peptidases (Step 8) (11).

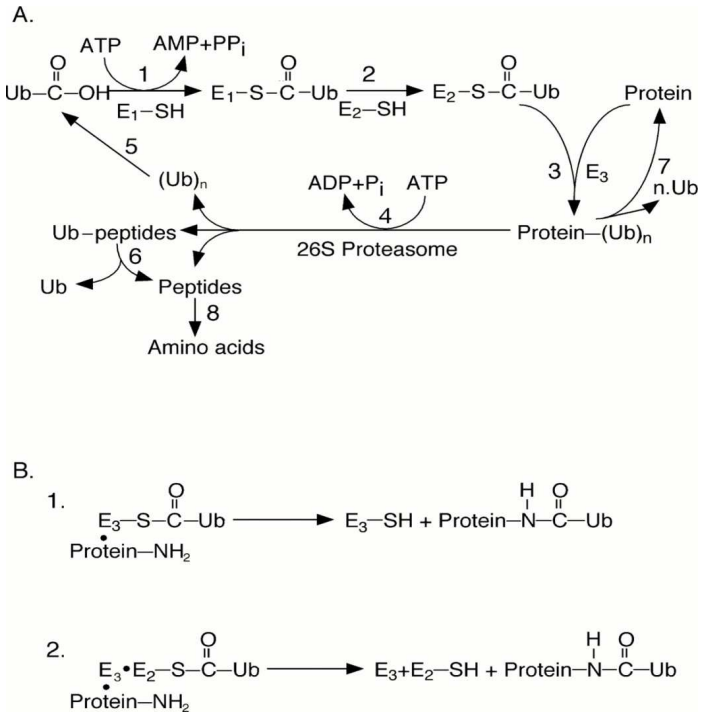


Fig. 1.3 Enzymatic reactions of the ubiquitin system

A. Sequence of reactions in the proteolytic pathway.

B. Possible mechanisms of ubiquitin transfer by different types of E3 enzymes

Different types of E3s may carry out the transfer of ubiquitin to the substrate protein by two different mechanisms. In some cases, such as with the Hect-domain family of E3 enzymes (explain in Chapter 1.3), ubiquitin is first transferred from an appropriate E2 to an active site Cys residue of the E3 enzyme. This E3-ubiquitin thiolester is the donor for amide bond formation with the protein substrate (Step 1B). In other cases E3 enzymes bind E2s tightly and they also bind their appropriate protein substrate, ubiquitin can be transferred directly from E2 to the protein substrate (Step 2B) (11) .

The rapid degradation of ubiquitinated proteins is catalyzed by the 26S proteasome. This structure is found in the nucleus and the cytosol of all cells and constitutes approximately 1 to 2% of cell mass (10). The functional large 26S proteasome assembles from ring-shaped 19S and 20S particles, which are composed of numerous polypeptide subunits. Peptidases located in the subunits of the proteasome then digest the substrate, releasing peptide fragments (6). The 19S caps cleave off the ubiquitin chain and use ATPases to unfold the

protein substrate and translocate it into the 20S core particle for degradation into peptides (10) (fig 1.4).

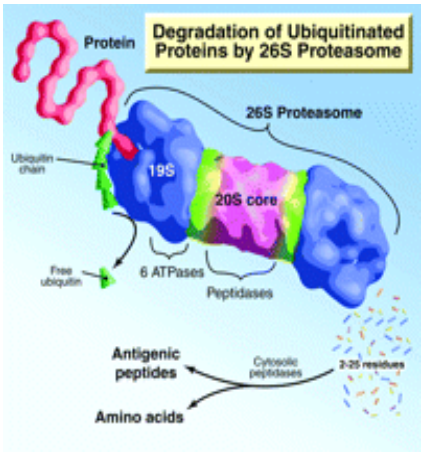


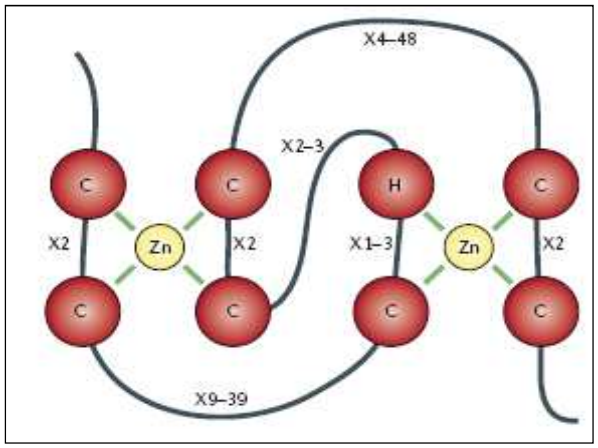
Fig. 1.4 26S proteasome

1.3 E3 ubiquitin ligases

E3 ubiquitin ligases represent a very large number of protein molecules (up to 600 in the human genome) which fall into two classes based on common sequence motifs and catalytic mechanism. The two protein classes identified for E3 ubiquitin ligases are and the HECT-domain containing proteins the RING-finger like proteins (12).

HECT domain (homologous to E6-AP carboxy-terminus) proteins are large monomeric E3s that consist of two functionally distinct domains. The C-terminal HECT domain (350 amino acids) accepts the activated ubiquitin from the E2s by forming a thiolester linkage with ubiquitin, enabling it to be transferred to the substrate. HECT-domain E3s directly bind activated ubiquitin and are actual components of the enzymatic conjugation cascade. The prototypical member of this family is the E6-associated protein (E6-AP) (10). The E6-A5 E3 ligase was the first one to be described in targeting p53 for ubiquitinylation. This process in initiated in the presence of E6, the gene product of HPV-16, so use of this type of ligase is limited to cancers with causative links to oncogenic HPV infections, such as cervical carcinomas (6).

The RING finger protein sequence motif was first identified in the protein product of the human gene RING1 – Really Interesting New Gene 1 – which is located on chromosome 6.



The RING finger can be defined as unique linear series of conserved cysteine and histidine residue and binds two zinc atoms in a unique cross-brace arrangement (13) (fig 1.5).

RING finger proteins can be either monomeric or members of multisubunit E3 ligase complexes (6). Single-subunit E3s contain the substrate recognition element and the RING finger on the same polypeptide. The well studied E3s include the oncoprotein Mdm2, which ubiquitylates p53 and the protooncoprotein c-Cbl, which ubiquitylates growth factor receptors (7). The BRCA1 RING E3 ligase plays a role in DNA repair and for this reason it´s important as a tumor suppressor. BRCA1 mutations in its N-terminal RINF-finger are associated with familial breast and ovarian carcinoma (6). Selected single-subunit RING finger E3s are in fig1.6.

TABLE 3 Selected single-subunit RING finger E3s

Name	**Cognate E2**	**Ubiquitination signal**	**Substrate(s)/Properties**
Higher organisms			
E3α/Ubr1	UbcH1	Type 1, 2 N-terminal amino acids	Several E3 isoforms
E3α/Ubr1	UbcH1	LLVRGRTLVV	Substrate: EMC 3C protease
BRCA1/2			Role in DNA repair and transcriptional control as tumour suppressor
Rad18	UbcH1	Unknown	Function: postreplication repair
c-Cbl	UbcH7, H5B	Phosphotyrosine	Substrates: EGF and PDGF
Mdm2	Unknown	Includes dephospho	Substrate: p53
Parkin	Unknown	Unknown	Mutated in Parkinson's disease
Siah-1,2	Unknown	Unknown	Substrate: deleted in colorectal cancer
IAPs	UbcH5B	Unknown	Substrate: self; functions in
			regulation of apoptosis
TRAF6	Ubc13/Uev1A	Unknown	Functions in IKK activation

Fig 1.6

Multisubunit E3s include a small RING finger protein and a protein from the CULLIN family (7) (fig1.7).

Table 1 | **Multisubunit, Cullin-containing RING E3s***

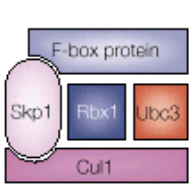
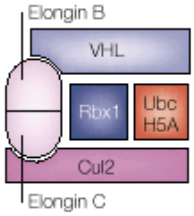
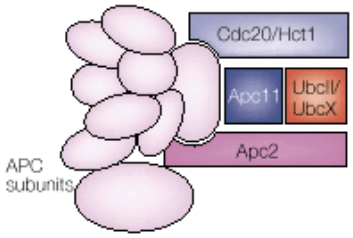
	SCF	VCB-CUL2	APC
			
RING	Rbx1 (Hrt1/Roc1)	Rbx1 (Hrt1/Roc1)	Apc11
Cullin	Cdc53 (Cul1)‡	Cul2‡	Apc2
Adaptors	Skp1	Elongin B: homologous to amino terminus of Skp1. Elongin C: a UDP.	Multiple APC subunits (pink), some with tetratricopeptide repeats. These presumably have adaptor functions.
E2	Ubc3 (Cdc34)	UbcH5A, others?	Ubc11, UbcX
Substrate recognition	F-box proteins. These include those with WD40 repeats, leucine-rich domains and others.	VHL, possibly other SOCS box-containing proteins.	Cdc20 (Fizzy) and Hct1 (Fizzy-related); both contain WD40 repeats.
Substrates (partial list)	Sic1, IκBα, β-catenin, G1 cyclins, CD4 bound to phosphorylated HIV Vpu, others.	HIF1α	Mitotic cyclins, Pds1, Cut2, Ase1, Scc1, Securin, others.

Fig 1.7

The SCF (Skp1-Culin-F-box protein) complexes ubiquitinylate a diverse group of substrates involved in cell cycle progression, signal transduction and transcription. The variable F-box protein subunit binds the substrate to be ubiquitinated. Deregulation of SCF-dependent proteolysis can contribute to neoplastic transformation; because key proteins are know to be modulated by SCF complex (6).

The APC (anaphase-promoting complex) includes at least 12 subunits. The important regulators of APC activity are phosphorylation and dephosphorylation (7).

1.4 BCA2

BCA2 is a novel RING-type ubiquitin ligase and was identified in the laboratory of Prof. Angelika Burger from among 950 cDNAs isolated by subtractive hybridization and differential display cloning techniques using normal and breast cancer cell lines RNAs (5).

The full-length BCA2 gene was cloned from the human breast cancer cell line; it encodes an open reading frame of 304 amino acids and contains a RING-H2 domain. The autoubiquitination activity of BCA2 indicates that it is a novel RING-type E3 ligase (5).

BCA2 RNA is expressed at extremely low levels in normal tissues. In contrast, much higher BCA2 levels are seen in breast and prostate cancer cell lines with the ER-positive cell line MCF7 having the highest BCA2 RNA expression. In a group of 945 invasive breast cancer cases 56% were found overexpressed BCA2 in nucleus and cytoplasm. This overexpression is significantly correlated with positive estrogen receptor status (5).

BCA2 is a target protein of Rab7 (a member of the Rab family of small G proteins). Rab GTPases play key roles in regulating vesicle trafficking in exocytic and endocytic pathways. The model of BCA2 and Rab7 interactions is shown in figure 1.8. Receptor–mediated endocytosis activated EGF-R complexes and transports them to the early endosome of sorting. Rab7 GTPases regulate vesicle fusion events during this process (5).

The mouse homologue of BCA2 has been called Rabring7 (5), Rab7-interacting RING finger protein. It contains an H2 type RING finger motif at the C termini. Rabring7 specifically binds the GTP-bound form of Rab7 at the N-terminal portion. Rabring7 is found mainly in the cytosol and is recruited efficiently to late endosomes/lysosomes by the GTP-bound form Rab7 and plays crucial roles as a Rab7 target protein in vesicle traffic to late endosome/lysosome and lysosome biogenesis (14).

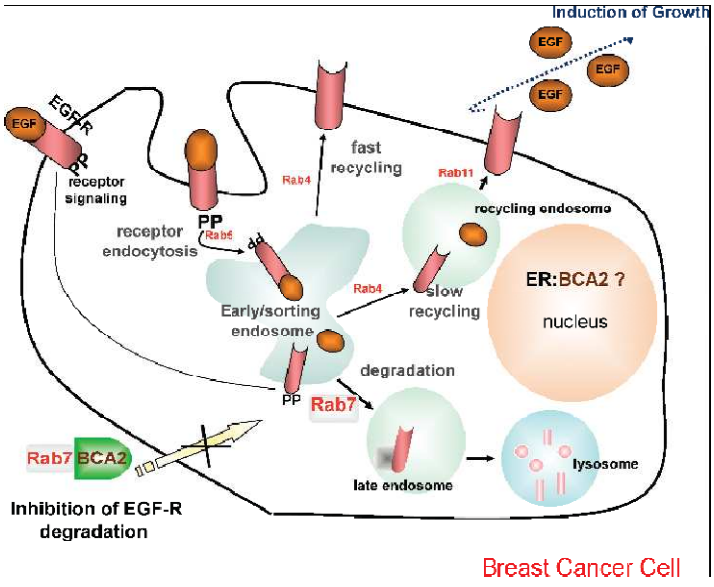


Fig1.8 Model of BCA2 and Rab7 interactions of intracellular vesicle trafficking in breast cancer

The role of the RING-H2 finger and its zinc-complexing structure for BCA2 and other RING E3 ligases is underscored by the fact that the zinc-ejecting compound disulfiram is able to inhibit BCA2 autoubiquitination. If analogs with retained or even enhanced zinc ejecting capabilities but without ALDH inhibition could be developed, they might prove valuable for the treatment of breast cancer (15).

1.5 BCA2 inhibitors

Disulfiram has been used for over half a century for alcohol aversion therapy (16) as an alcohol dehydrogenase inhibitor and is also known for its zinc-ejecting properties. Most recently, disulfiram was been described to inhibit angiogenesis and invasion of human tumor cells (5). The line of investigation of new BCA2 inhibitors is currently being undertaken at the Welsh School of Pharmacy (see fig1.9)

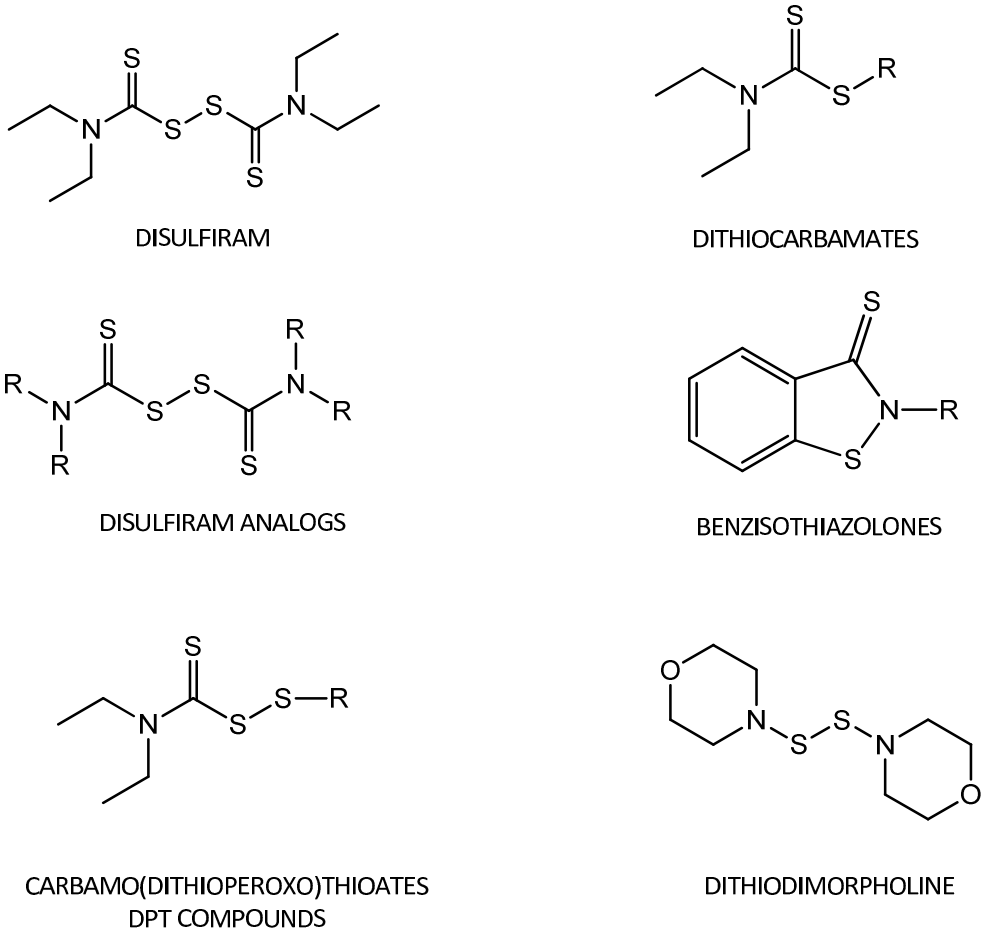


Fig1.9 BCA2 inhibitors

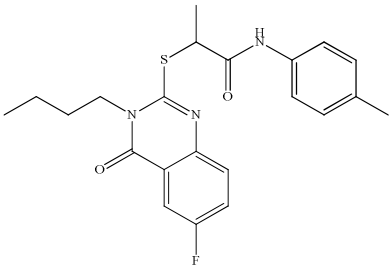
Several zinc-ejecting agents have been shown to very specifically target zinc and RING finger proteins, so it is possible that disulfiram analogs could be developed and provide a novel approach for the treatment of breast cancer.

Chapter 2

Project Aims

The aim of this project is try to successfully synthesise three types of derivatives.

2.1 Quinazoline derivatives



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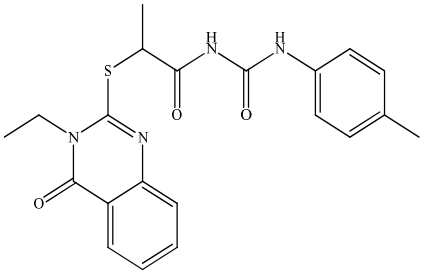

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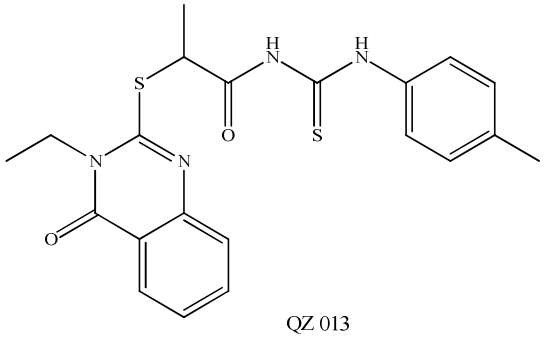

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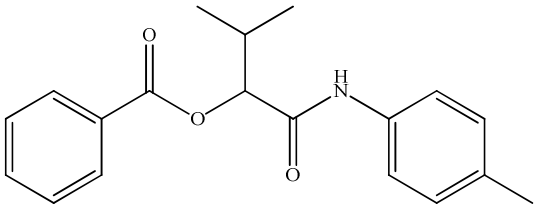
2.2 Derivatives of 2-amino-2-oxoethyl-benzoate



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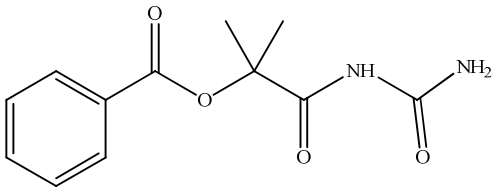

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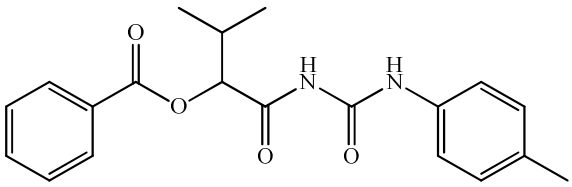

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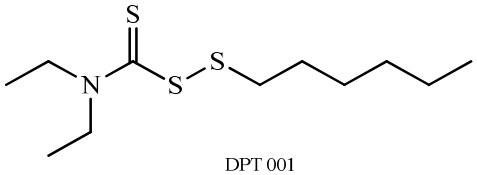
2.3 DPT compounds



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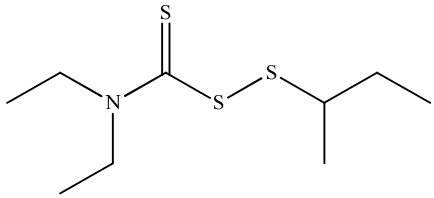

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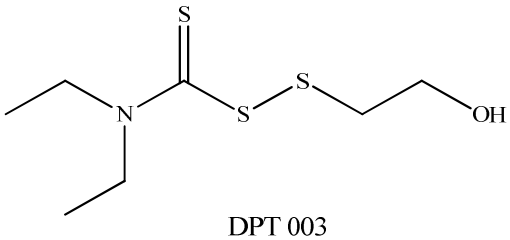

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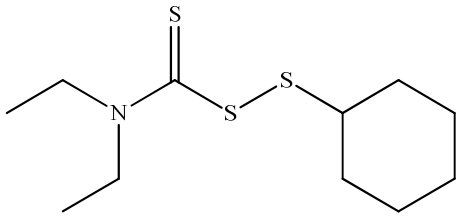
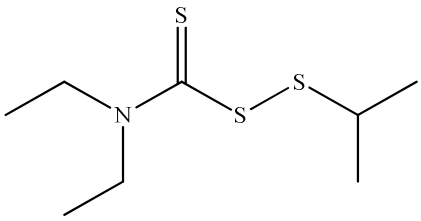
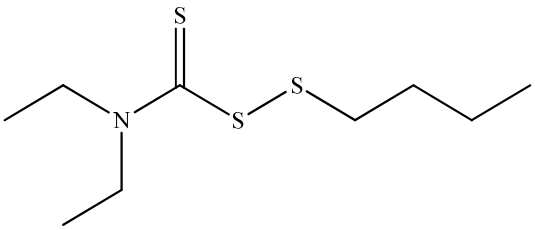


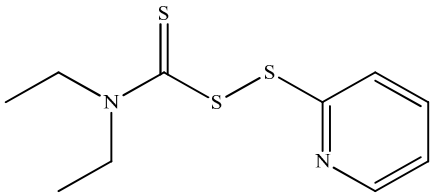
DPT 004



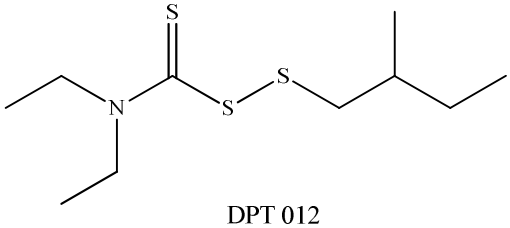
DPT 005



DPT 006



DPT 011



DPT 012

After these compounds have been synthesised they will be sent to the Angelica Burger group (Barbara Ann Karmanos Cancer Institute, Wayne State University, Detroit, MI) to be tested for BCA2 inhibitory activity.

Chapter 3

Method

3.1 Synthesis plan for Quinazoline derivatives

The synthesis of QZ products was accessed by the formation of a thio-ether bond between fragments (a) and (b) through an SN2 type reaction. Figure 3.1 illustrates a three steps synthesis of final compound (c).

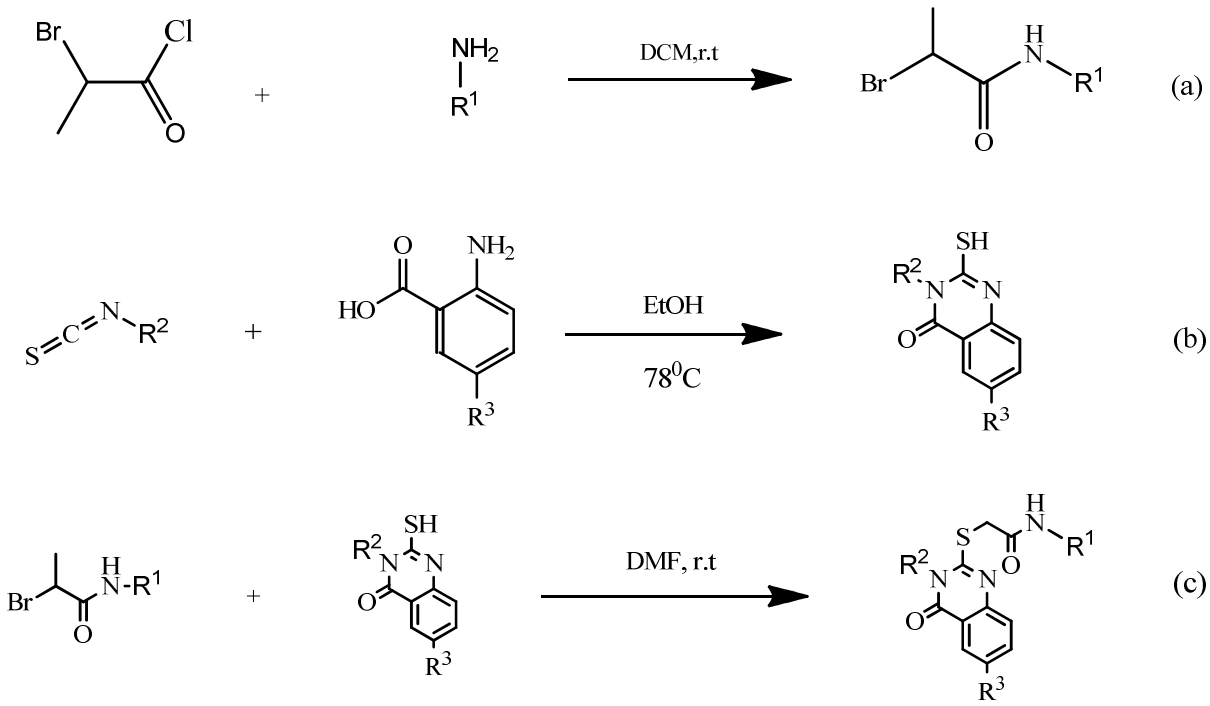
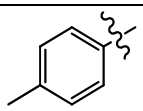
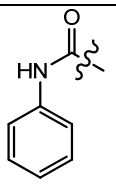
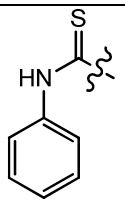
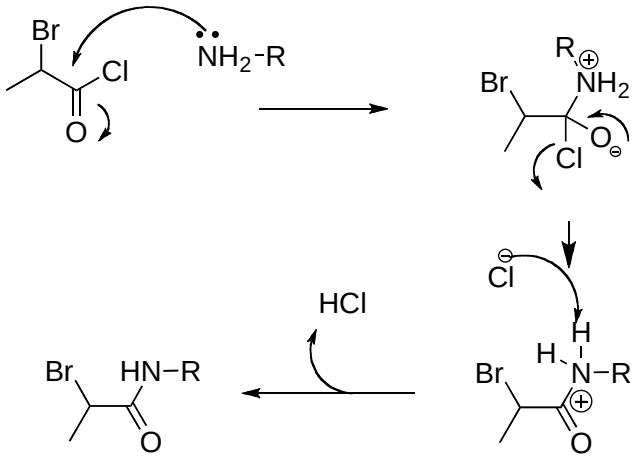


Fig3.1

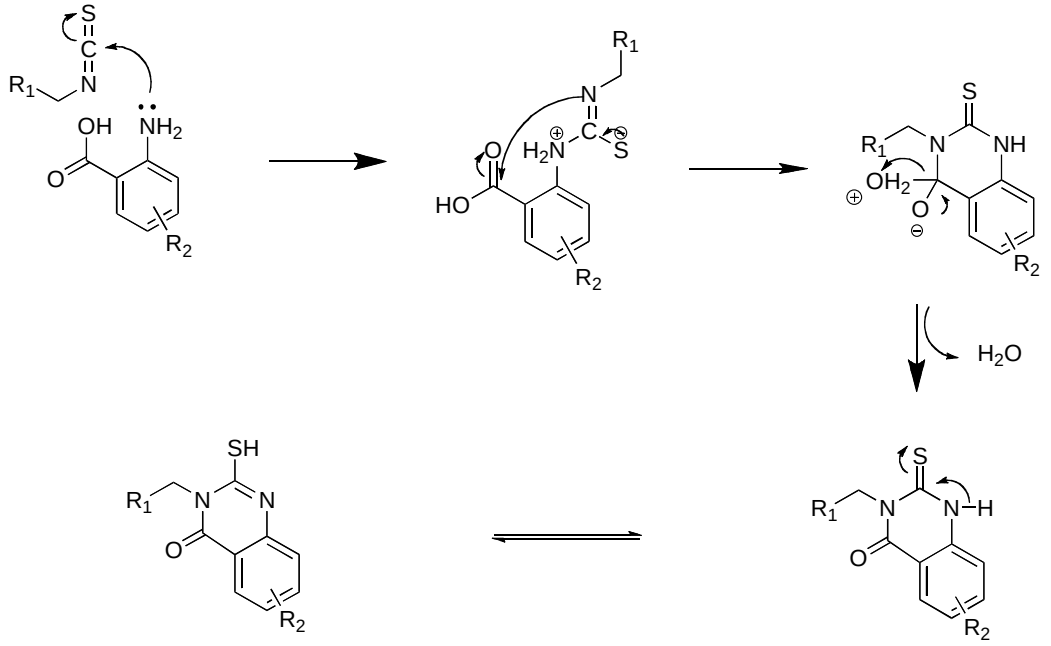
	R^1	R^2	R^3
QZ 011		C_4H_9	F
QZ 012		C_2H_5	H
QZ 013		C_2H_5	H

DMF was used as polar and aprotic solvent, which provides the bimolecular nucleophilic substitution. Potassium carbonate anhydrous was used as base to activate the thiol group in fragment (b). In most cases, compounds were collected by filtration after pouring the reaction mixtures into ice water. The synthesis of fragment (a) is a bimolecular nucleophilic substitution in which a lone pair on the N of the amine group attacks carbonyl of 2-bromopropanoylchloride. The last step involves the removal of the proton by the chloride ion. Mechanism of this reaction is shown in scheme 1.1



Scheme 1.1

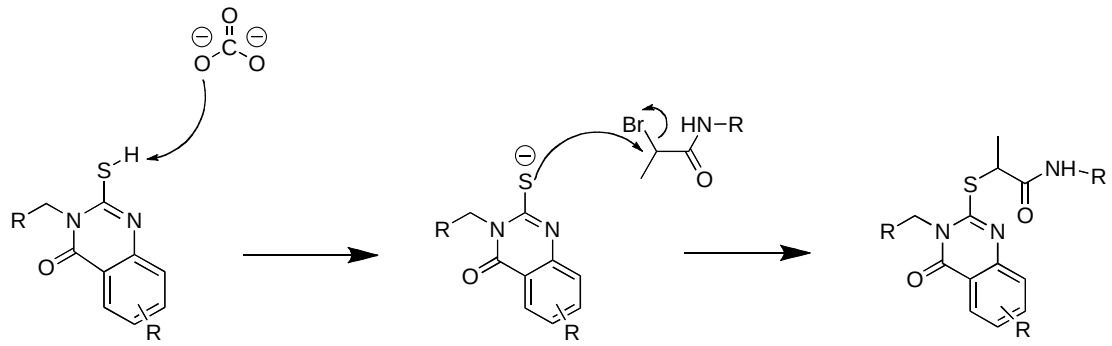
The mechanism of the syntesis fragment (b) is shown in scheme 1.2. At first the lone pair of primary amine group attacks the carbon of isothiocyanate group. Then nucleophilic attack occurs between the amine group and carbonyl group of the same compound. The cyclisation is finished by elimination of water.



Scheme 1.2

For synthesis the final compound (c) was used at first potassium carbonate as a base to activate thiol group of fragment (b). Thiol group attacks carbon of fragment (a) and Br is eliminated from fragment (a).

Mechanism of this reaction is shown in scheme 1.3.



Scheme 1.3

3.2 Synthesis plan for derivatives of 2-amino-2-oxoethyl-benzoate

3.2.1 JS 001

The final compound JS 001 was planned to be synthesize by the formation of ester bond between fragment (a) and benzoic acid through an SN2 type reaction. DMF was used as polar and aprotic solvent, which provides the bimolecular nucleophilic substitution. Sodium hydrine was used as a base to activate the carboxylic group in fragment (a). Figure 3.2 illustrates a two steps synthesis of final compound JS 001.

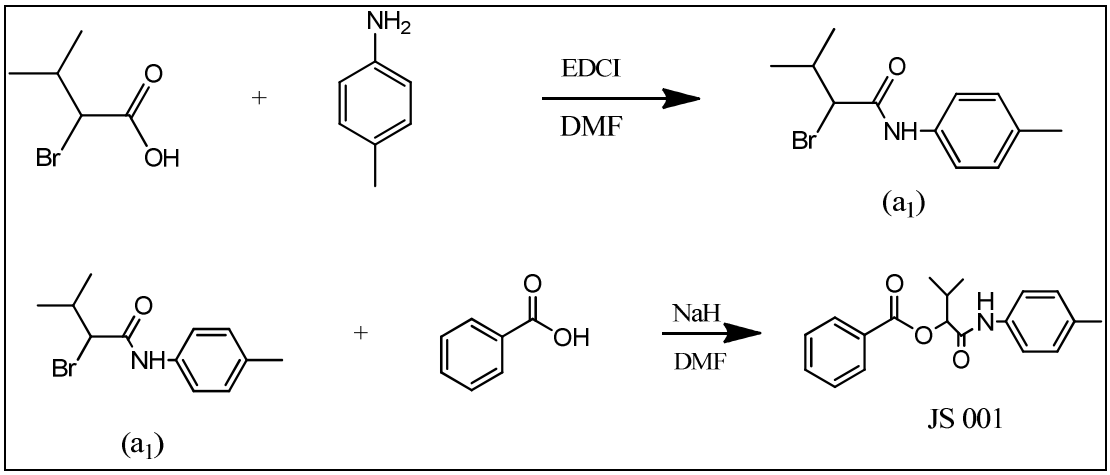
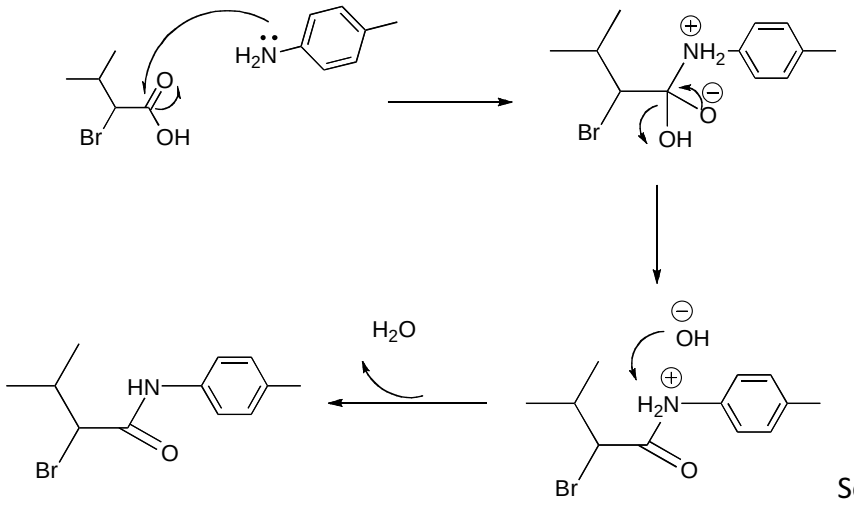


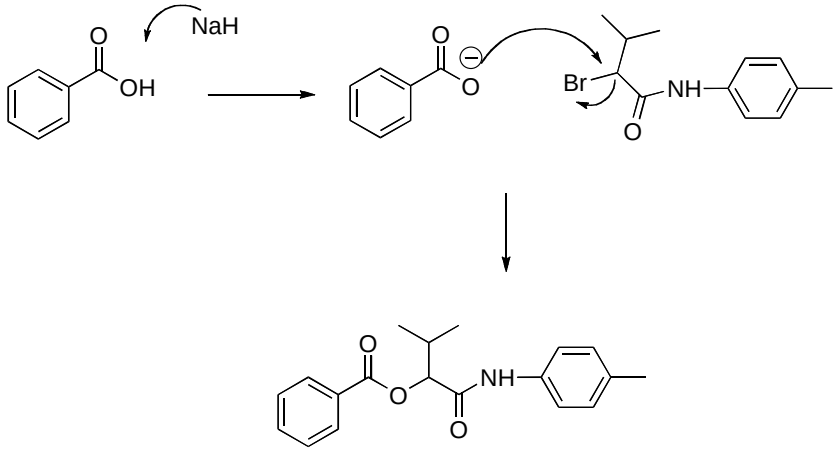
Fig3.2

The synthesis of fragment (a) is a bimolecular nucleophilic substitution in which a lone pair on the N of the secondary amine group attacks carbonyl of 2-bromo-3-methylbutanoic acid. EDCI was used to relieve the amide formation between carboxylic acid and amine. This method was derived from an article about mechanism of amide formation (17). The last step involves the elimination of water. Mechanism of this reaction is shown in scheme 1.4.



Scheme1.4

For synthesis final compound JS 001 was used at first sodium hydrine as a base to activate carboxylic group of benzoic acid. The reaction was accessed in dry condition in dry DMF. Forming ester bond and elimination of Br has finished the reaction. Mechanism of this reaction is shown in scheme 1.5.



Scheme1.5

3.2.2 JS 002

For synthesis JS 002 was been used two steps synthesis (illustrates in fig3.3). In the first step was used silver oxide, which relieves the formation of ester bond. This reaction was derived from an article about a direct functionalization of tertiary alkyl bromides (18). Then

the fragment (b) was treated with 1-chloro-2-isocyanatoethane and Zn dust to provide final compound JS 002. Figure 3.3 illustrates a two steps synthesis of final compound JS 002.

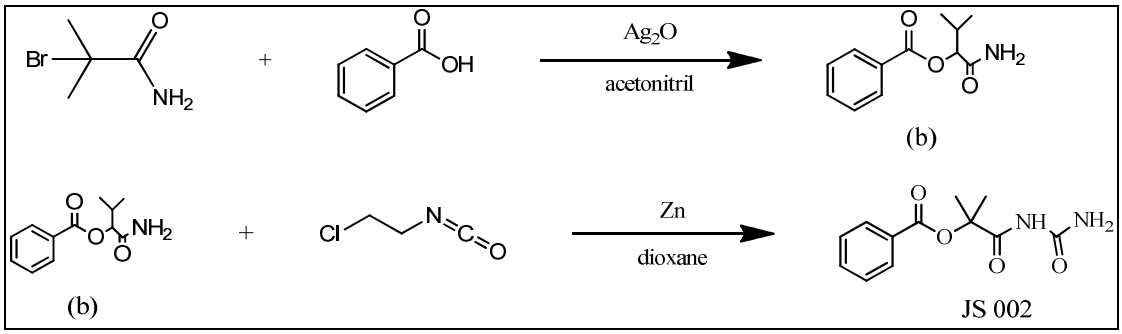
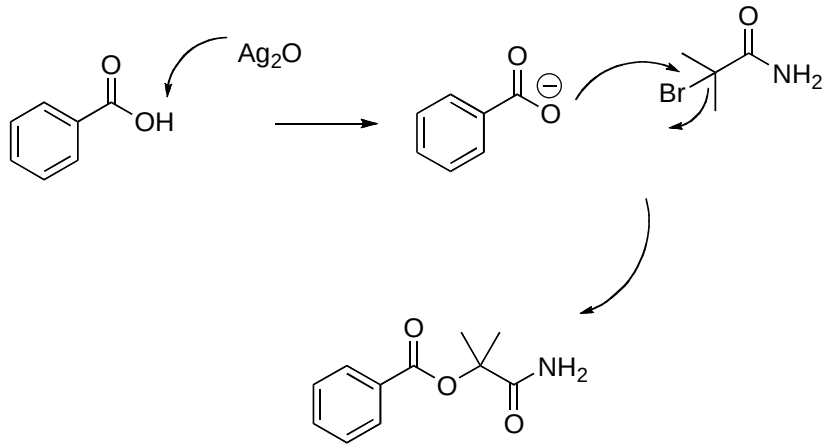


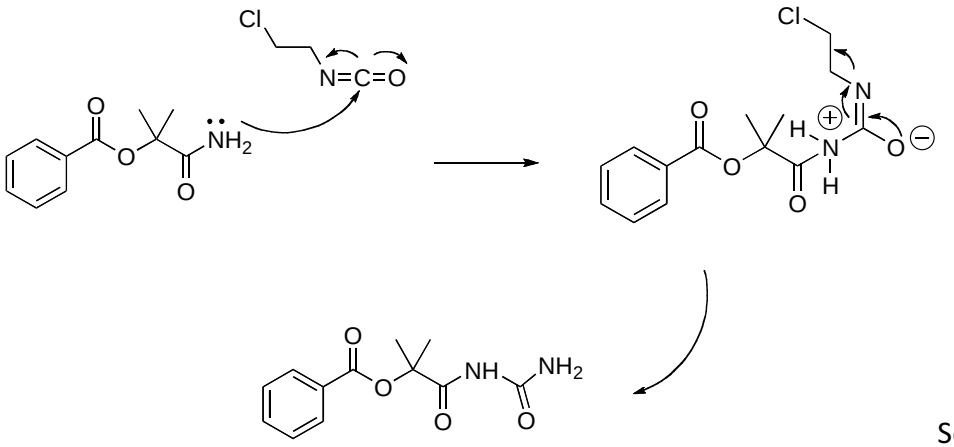
Fig3.3

The synthesis of fragment (b) is nucleophilic aromatic substitution using silveroxide in acetonitril/water (in combination 95/5). The mechanism of this reaction is shown in scheme 1.6.



Scheme1.6

The synthesis of final compound JS 002 was derived from an article which use for synthesis 1-chloro-2-isocyanatoethane and Zn dust[20].The mechanism of this reaction is shown in scheme 1.7.



Scheme1.7

3.2.3 JS 003

The synthesis of JS 003 was accessed by formation of ester bond between fragment (c_1) and benzoyl chloride in dry DCM. Figure 3.4 illustrates a two steps synthesis of final compound JS 003.

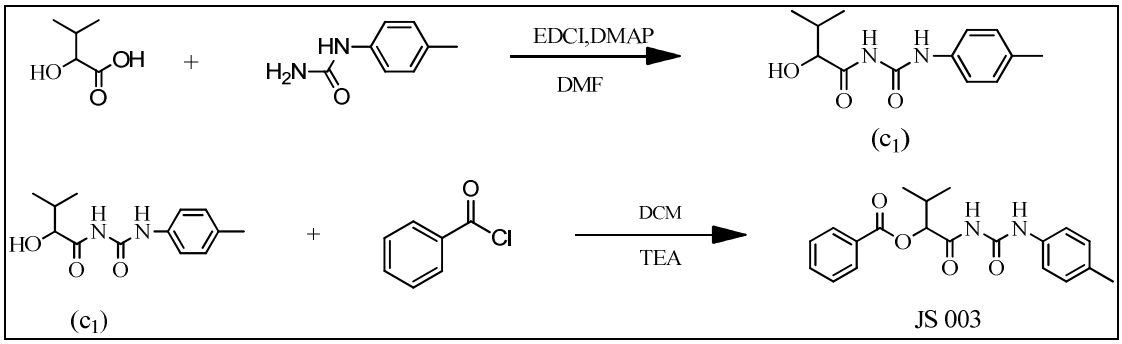
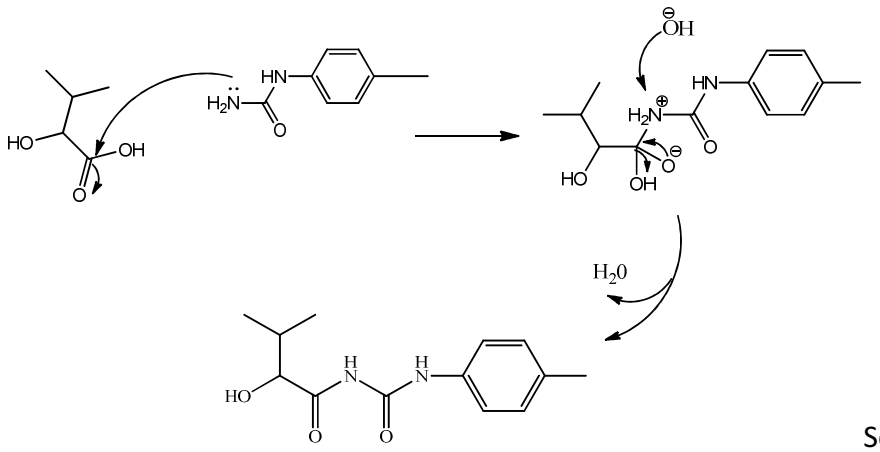


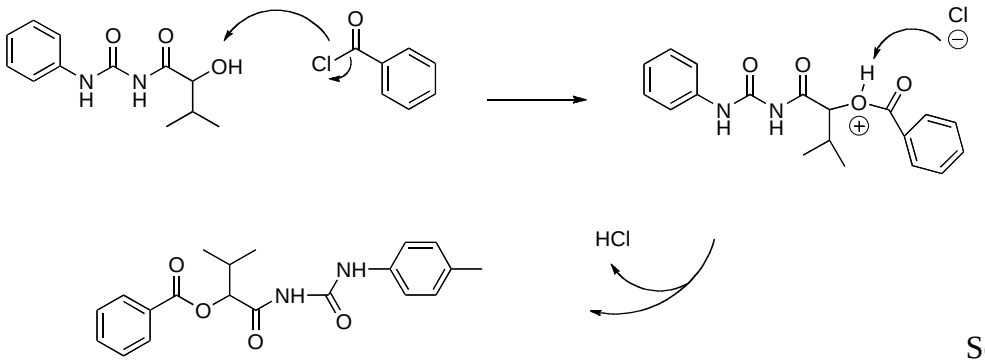
Fig3.4

The synthesis of fragment (c_1) is a bimolecular nucleophilic substitution in which a lone pair on the N of the secondary amine group attacks carbonyl of hydroxyisovaleric acid. EDCI was used to relieve the amide formation between carboxylic acid and amine. This method was derived from an article about mechanism of amide formation (17). DMF was used as polar and aprotic solvent, which provides the bimolecular nucleophilic substitution. The reaction is finished by elimination of water. The mechanism of this reaction is shown in scheme 1.8.



Scheme 1.8

The second step reaction was accessed in dry condition in dry DCM. TEA was used as a base. The reaction was finished by elimination of HCl. The mechanism of this reaction is shown in scheme 1.9.



Scheme 1.9

3.3 Synthesis plan for DPT compounds

The synthesis of DPT compounds was accessed in one step synthesis by reaction of disulfiram with appropriate thiol.

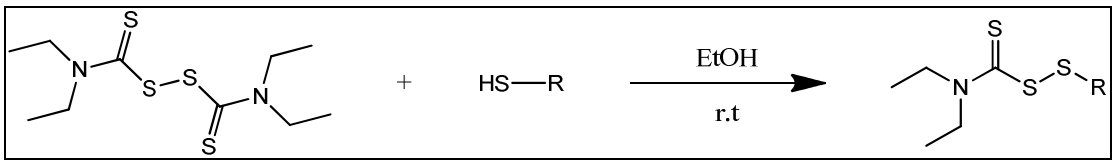


Fig3.5

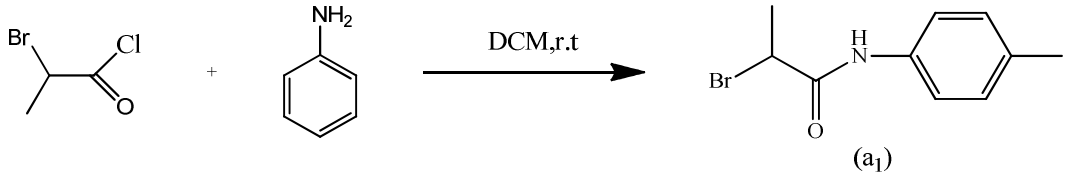
Chapter 4

Results and Discussion

4.1 Synthesis of Quinazoline derivatives

4.1.1 Synthesis of QZ 011

4.1.1A Synthesis of 2-bromo-*N*-*p*-tolylpropanamide - fragment (a_1)

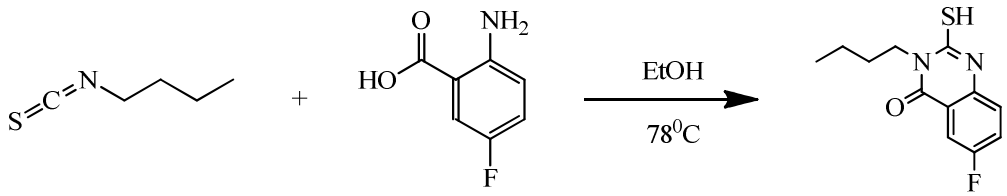


(a_1)

To synthesis fragment (a_1) was used an equimolar mixture of 2-bromopropanoylchloride and p-toluidine in DCM. Mechanism of this reaction is in scheme 1.1.

The reaction was monitored by TLC. After 20 minutes TLC confirmed that all starting material was converted into product. No purification of the product was done. Evaporation of the solvent gave a product as a yellow solid. The signals obtained from ^{1}H NMR analysis for this compound were representative of the structure and confirmed that the product had been successfully synthesized. The full NMR data of this compound are in section 6.2.1A.

4.1.1B Synthesis of 3-butyl-6-fluoro-2-mercaptoquinazolin-4 (3*H*)-one – fragment (b_1)

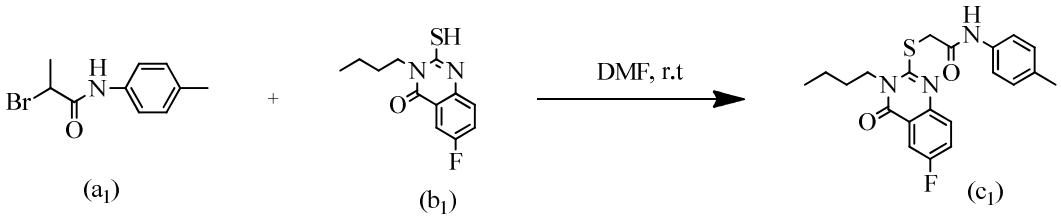


(b_1)

Fragment (b_1) was obtained by cyclisation reaction of 1-isothiocyanatobutane with equimolar amount of 2-amino-5-fluorobenzoic acid in EtOH.

This reaction was performed two times with yields 19% and 55%. The reaction was complete after 24 hours of reflux and was monitored by TLC. After this time the reaction turned cloudy with formation of white precipitate. More precipitate was induced by cooling reaction to room temperature and then cooling to 5°C. The precipitate was collected by filtration and dried in the fume-hood. ^{1}H NMR analysis of the product confirmed that the product had been synthesized. Full NMR data of this compound are in section 6.2.1B.

4.1.1C Synthesis of 2-(3-butyl-6-fluoro-4-oxo-3, 4-dihydroquinazolin-2-ylthio)-*N*-*p*-tolylpropanamide – final compound QZ 001 (c_1)



(a_1) (b_1) (c_1)

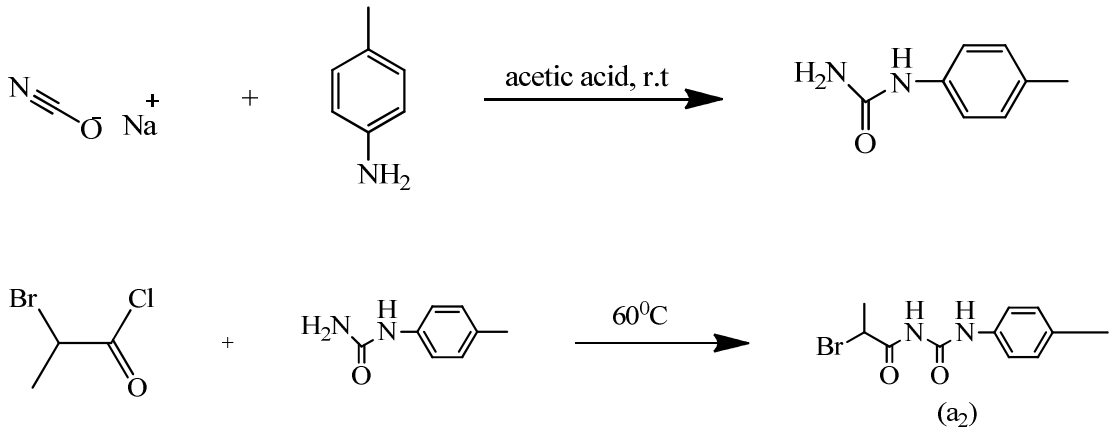
Final compound (c_1) was synthesized by reacting fragment (a_1) with equimolar quantity of fragment (b_1). At first potassium carbonate was used as a base to activate thiol group of fragment (b_1). Then thiol group attack carbon of fragment (a_1) and Br was eliminated from fragment (a_1).

This reaction was performed in dry conditions overnight and was monitored by TLC. When the starting material disappeared the reaction mixture was poured into ice-water, which induced forming of white precipitate. This precipitate was collected by filtration and dried. The yield of the reaction was 52%. The NMR data confirmed that the obtained product is QZ 001. The melting point is 133°C. The full NMR data of this compound are in section 6.2.1C.

4.1.2 Synthesis of QZ 012

4.1.2A Synthesis of 2-bromo-N-(p-tolylcarbamoyl)propanamide – fragment (a2)

The synthesis of fragment (a_2) was carried out in two steps. The first one involved the synthesis of *p*-tolyl-urea which was accessed by the reaction of *p*-toluidine with sodium cyanate. This latter was reacted with 2-bromo-proponyl-chloride in neat condition.



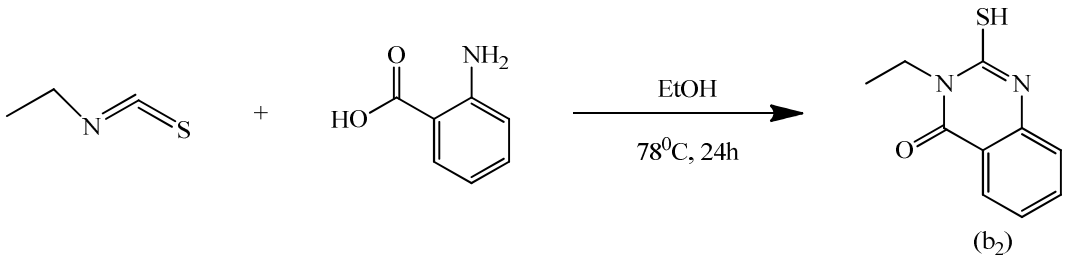
The mechanism of this reaction is described in scheme 1.1.

The method for synthesis p-tolyl-urea was derived from an article about mechanism of the basic hydrolysis of phenylureas (19) (20). This reaction was performed in acidic medium of acetic acid at r.t. After approximately 30 minutes p-tolyl-urea precipitated as a white solid and was collected by filtration. The full NMR data of this compound are in section 6.2.2A.

P-tolyl-urea isn´t soluble in any solvent, for that reason next reaction with 2-bromopropanoyl chloride was performed without solvent. The reaction generated HCl, which was monitored by pH papers. When it stopped, the reaction mixture was diluted with DCM. TLC confirmed that all starting material converted to product. Evaporation of the solvent gave a brown, sticky product with yield 65%. The full NMR data of this compound are in section 6.2.2B.

4.1.2B Synthesis of 3-ethyl-2-mercaptoquinazolin-4(3*H*)-one – fragment (b_2)

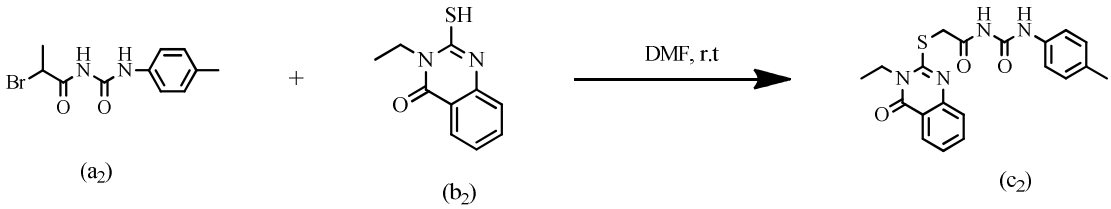
Fragment (b_1) was obtained by cyclisation reaction of 1-isothiocyanatoethan with equimolar amount of 2-aminobenzoic acid in EtOH. The mechanism of this reaction is shown in scheme 1.2.



The reaction was stirred at 78°C overnight in dry conditions. After 12hours TLC confirmed that all starting material disappeared. The product was retrieved by pouring reaction mixture into ice-water and filtration of the created precipitate. The yield was 57%.

4.1.2C Synthesis of 2-(3-ethyl-4-oxo-3,4-dihydroquinazolin-2-ylthio)-*N*-(*p*-tolylcarbamoyl)propanamide – final compound QZ 012 (c_2)

Final compound (c_1) was synthesised by reacting fragment (a_1) with equimolar quantity of fragment (b_1). The mechanism of this reaction is described in section in scheme 1.3. The full NMR data of this compound and the full procedure are in section 6.2.2D.



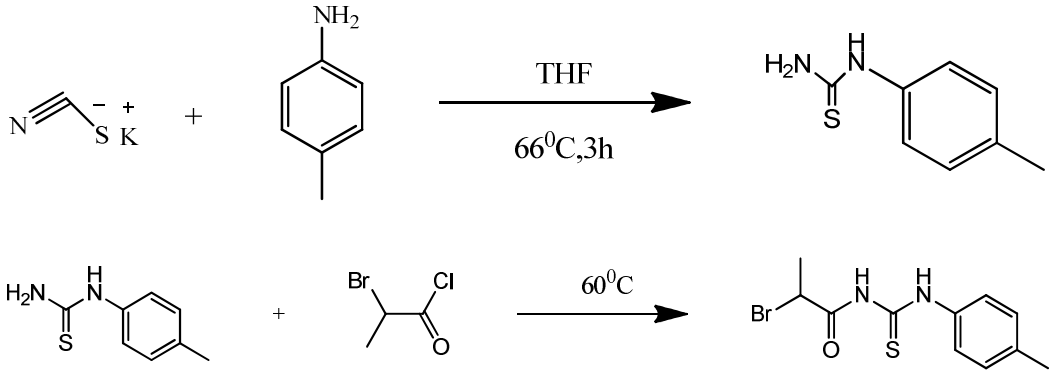
This reaction was performed in dry conditions overnight and was monitored by TLC. When the starting material disappeared the reaction mixture was poured into ice-water, which induced forming of white precipitate. This precipitate was collected

by filtration and dried. The yield of the reaction was 26%. The NMR data confirmed that the obtained product is QZ 012.

4.1.3 Synthesis of QZ 013

4.1.3A Synthesis of 2-bromo-*N*-(*p*-tolylcarbamothioyl)propanamide (fragment a_3)

The synthesis of fragment (a_3) was carried out in two steps. The first one involved the synthesis of 1-p-tolylthiourea, which was then reacted with 2-bromopropanoyl chloride.

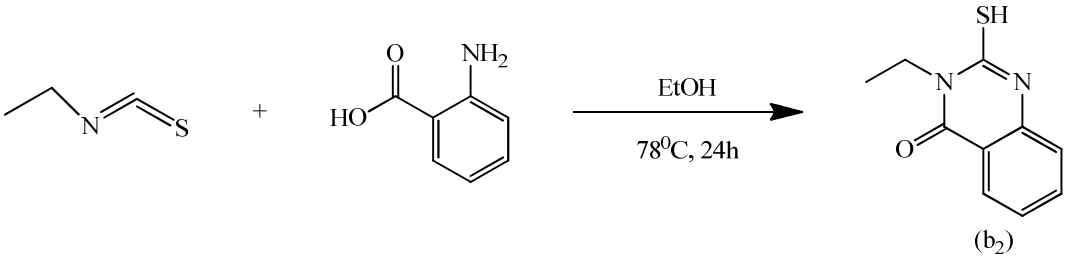


At first I synthesized 1-p-tolylthiourea in a same way like synthesis p-tolyl-urea. An equimolar mixture of potassium thiocyanate and p-toluidine in medium of acetic acid was stirred at room temperature, but no product appeared. The next procedure to synthesize 1-p-tolylthiourea was derived from an article about a convenient method for the preparation of primary and symmetrical N,N'- disubstituted thioureas (20). To a stirred solution of p-toluidine HCl in THF was added potassium thiocyanate. P-toluidine HCl isn't completely soluble in THF. After adding potassium thiocyanate the mixture changed the color and was purple. The mixture was heated at reflux for 24 hours at 66^0C. The reaction was monitored by TLC, when the starting material disappeared; the mixture was diluted with water and washed with EA, then with HCl and brine. The obtained organic phase was dried and evaporation of the solvent gave a product as a yellow solid. The yield of this reaction was 41%. The NMR data and full procedure of this product are in section 6.2.3A.

The second step of synthesis involved the synthesis of fragment a_3 which was accessed by reaction of equimolar mixture of 1-p-tolylthiourea with 2-bromopropanoyl chloride without any solvent. The reaction generated HCl, which was monitored by pH papers. When it stopped, the brown reaction mixture was diluted with DCM. The TLC showed three spots. By adding EA to the reaction mixture the product precipitated, evaporation of solvent gave product as a yellow solid. ^{1}H NMR analysis of the product confirmed that the product had been synthesized. Full NMR data of this compound are in section 6.2.3B.

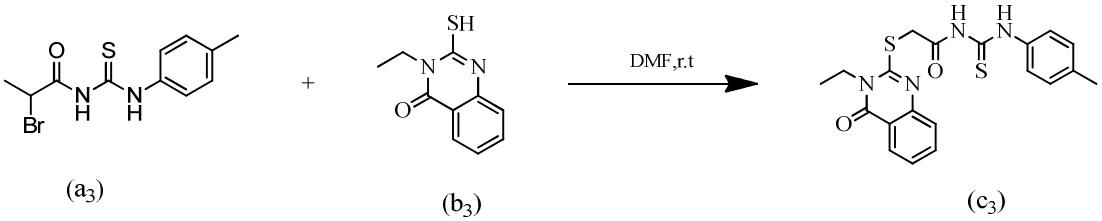
4.1.3B Synthesis of 3-ethyl-2-mercaptoquinazolin-4(3*H*)-one (fragment b_3)

Same compound as in 4.1.2B.



4.1.3C Synthesis of 2-(3-ethyl-4-oxo-3, 4-dihydroquinazolin-2-ylthio)-*N*-(*p*-tolylcarbamothioyl)propanamide – final compound QZ 003 (c_3)

Final compound (c_3) was synthesized by reacting fragment (a_3) with equimolar quantity of fragment (b_3). The mechanism of this reaction is described in scheme 1.3.



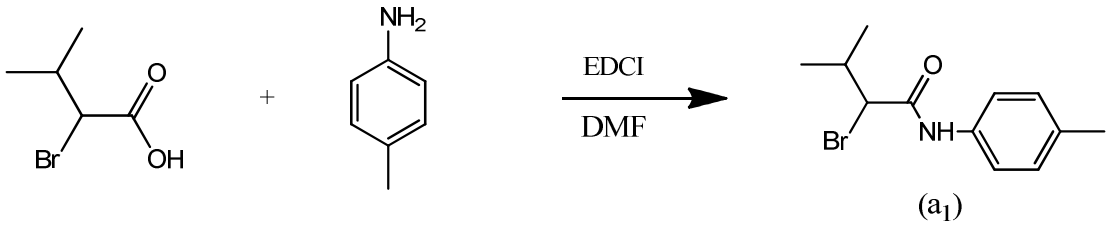
This reaction was performed two times. In both cases the obtained precipitate was starting material 3-ethyl-2-mercaptoquinazolin-4(3*H*)-one (fragment b_3).

4.2 Synthesis of derivatives of 2-amino-2-oxoethyl-benzoate

4.2.1 JS 001

4.2.1 A Synthesis of 2-bromo-3-methyl-*N*-*p*-tolylbutanamide – fragment (a_1)

To synthesize fragment (a_1) was used a mixture of 2-bromo-3-methylbutanoic acid, EDCI (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) and p-toluidine in anhydrous DMF. EDCI relieves the amide formation between carboxylic acid and amine. This method was derived from an article about mechanism of amide formation (17).



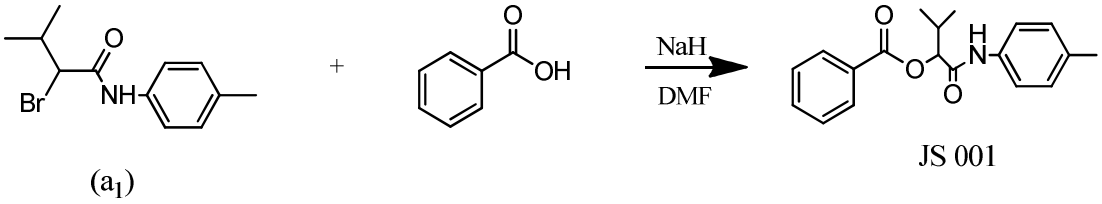
(a_1)

The mixture was stirred overnight, solvent was evaporated and the residue was dissolved in EA. This solution was washed with HCl. Then was concentrated and crystalizated. ^{1}H NMR analysis of the product confirmed that the product had been synthesized, but in a very little yield.

Next method for synthesis fragment (a_1) was derived from an article about synthesis and evaluation of nitrofuranylamides. For synthesis was used an equimolar mixture of 2-bromo-3-methylbutanoic acid and p-toluidine in anhydrous DMF. This mixture was treated with EDCI followed by DMAP. DMAP was used as a base. The resulting mixture was stirred in dry conditions for about 3 hours at r.t. The reaction was monitored by TLC. When the starting material disappeared the reaction mixture was poured into EA and washed with HCl and sodium carbonate. The evaporation gave a product as a solid with yield 50%. ^{1}H NMR analysis of the product confirmed that the product had been synthesized. Full NMR data of this compound are in section 6.3.1B.

4.2.1B - Synthesis of 3-methyl-1-oxo-1-(*p*-tolylamino) butan-2-yl-benzoate – JS 001

For synthesis of JS 001 was used equimolar mixture of 2-bromo-3-methyl-*N*-*p*-tolylbutanamide and benzoic acid. Sodium hydrine was added as a base. The reaction mixture was stirred in dry condition at r.t.



(a_1) JS 001

This reaction was performed three times with different conditions. In the first reaction an equimolar mixture of benzoic acid and sodium hydrine was stirred in dry condition for 30 minutes. Then was added 2-bromo-3-methyl-*N*-*p*-tolylbutanamide and the reaction mixture was stirred for 24 hours and monitored by TLC. The reaction mixture was poured into ice-water forming a precipitate. Recording to ^{1}H NMR analysis of the product, the obtained precipitate was starting material (a_1).

In the second reaction was an equimolar mixture of benzoic acid and sodium hydrine stirred in dry condition at r.t for 1 hour. Then the solvent was evaporated. Sodium benzoate obtained by this reaction was added to 2 equivalents of 2-bromo-3-methyl-*N*-*p*-tolylbutanamide and stirred at r.t for one week, then filtrated and dried in the fume hood. Recording to ^{1}H NMR analysis of the product, the obtained precipitate was starting material (a_1).

In the third reaction was stirred a mixture of 2-bromo-3-methyl-*N*-*p*-tolylbutanamide with 2 equivalents of benzoic acid in acetonitril and water. Later was added silver oxide. This reaction was derived from an article about a direct functionalization of tertiary alkyl bromides. The silver oxide promoted the direct functionalization, but only of tertiary alkyl bromides. In this reaction was used secondary alkyl bromide and for that reason no product was obtained.

This method wasn't successful, so next method for synthesis final compound JS 001 uses different starting material.

4.2.1C Synthesis of 2-hydroxy-3-methyl-*N*-*p*-tolylbutanamide – fragment (a_2)

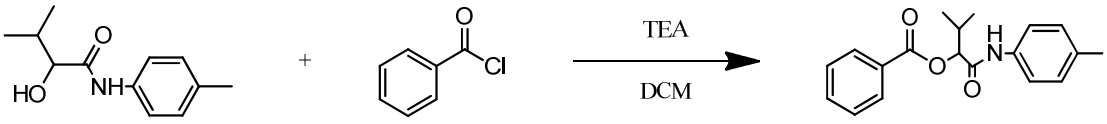
For synthesis fragment (a_1) was used an equimolar mixture of 2-hydroxy-3-methylbutanoic acid and p-toluidine in anhydrous DMF. This mixture was treated with EDCI followed by DMAP (4-Dimethylaminopyridine) like in synthesis of fragment (a_1). DMAP was used as a base.

(a_2)

The evaporation of reaction mixtue gave a product as a solid with yield 33%. ^{1}H NMR analysis of the product confirmed that the product had been synthesized. Full NMR data of this compound are in section 6.3.1D.

4.2.1D Synthesis of 3-methyl-1-oxo-1-(*p*-tolylamino) butan-2-yl-benzoate – JS 001

An equimolar mixture of 2-hydroxy-3-methyl-*N*-*p*-tolylbutanamide and benzoyl chloride was stirred in dry conditions for 12hours. TEA (1.1 equivalents) was added as a base.



(a_2) JS 001

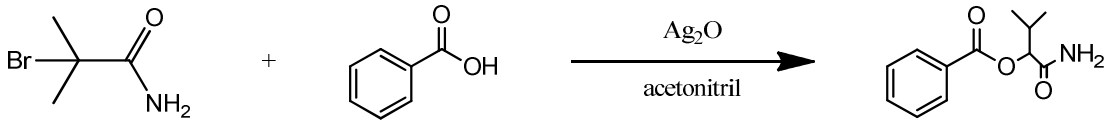
The reaction was monitored by TLC. There were three spots after 12 hours. Column purification (95%Hx, 5%EA) was used to purify the reaction mixture. The product came as a first spot with yield 10%. ^{1}H NMR analysis of the product

confirmed that the product had been synthesized. Full NMR data of this compound are in section 6.3.1E.

4.2.2 JS 002

4.2.2A Synthesis of 1-amino-2-methyl-1-oxopropan-2-yl-benzoate - fragment (b)

A mixture of benzoic acid and 2 equivalents of 2-bromo-2-methylpropanamide in acetonitrile in water was stirred at r.t. Silveroxide was added to a reaction mixture in one part. The reaction was stirred at r.t for 2 hours.

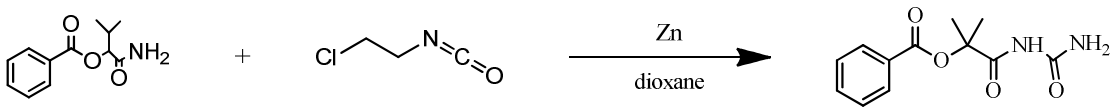


(b)

The reaction mixture was filtered through Celite, evaporation of the filtrate gave a product as a solid with yield 71%. ^{1}H NMR analysis of the product confirmed that the product had been synthesized. Full NMR data of this compound are in section 6.3.2A.

4.2.2B Synthesis of 2-methyl-1-oxo-1-ureidopropan-2-yl-benzoate – JS 002

1-amino-2-methyl-1-oxopropan-2-yl benzoate (fragment b) was dissolved in dioxane and treated with 1-chloro-2-isocyanatoethane. The reaction mixture was stirred for 3 hours at r.t. After addition of MeOH and Zn dust the reaction mixture was stirred for next 3 hours. Zn dust was removed by filtration through Celite and solvent was evaporated.



(b) JS 002

Evaporation gave a product as a solid with yield 448%. ^{1}H NMR analysis is in section 6.3.2B, but interpretation of this analysis is very difficult. The product was sent for mass spec analysis, which confirmed that the obtained product isn't JS 002.

4.2.3 JS 003

4.2.3A Synthesis of 2-hydroxy-3-methyl-*N*-(*p*-tolylcarbamoyl)butanamide – fragment (c)

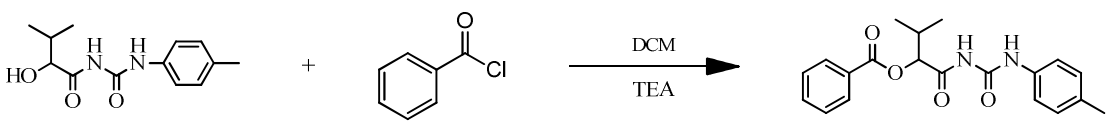
For synthesis fragment (c) was used an equimolar mixture of 2-hydroxy-3-methylbutanoic acid and 1-p-tolylurea. This mixture was treated with EDCI and DMAP in DMF. Same procedure as it is section 4.2.1A.

(c)

The evaporation of reaction mixture gave a product as a solid with yield 11%. ^{1}H NMR analysis of the product confirmed that the product had been synthesized. Full NMR data of this compound are in section 6.3.3A.

4.2.3B Synthesis of 3-methyl-1-oxo-1-(3-phenylureido)butan-2-yl-benzoate – JS 003

An equimolar mixture of 2-hydroxy-3-methyl-*N*-(*p*-tolylcarbamoyl)butanamide and benzoyl chloride was stirred at r.t in dry DCM in dry conditions. TEA was added as a base.

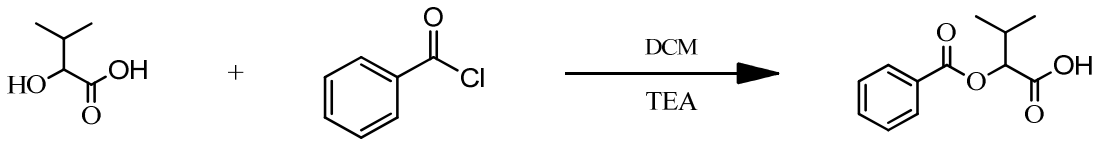


(c_1) JS 003

The reaction was monitored by TLC, but no product appeared.

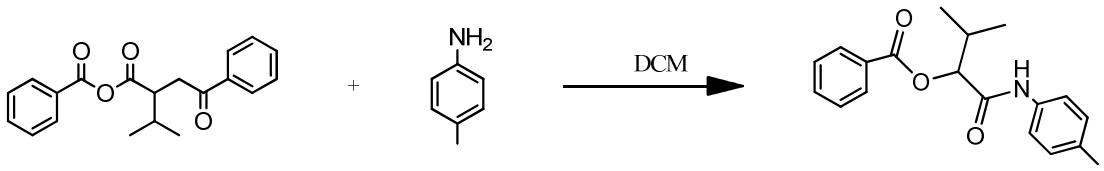
Synthesis of final compound JS 003 was then performed in different ways.

In the next reaction 2-hydroxy-3-methylbutanoic acid and equimolar amount of benzoyl chloride was stirred at r.t in dry DCM in dry conditions to give 2-(benzoyloxy)-3-methylbutanoic acid.

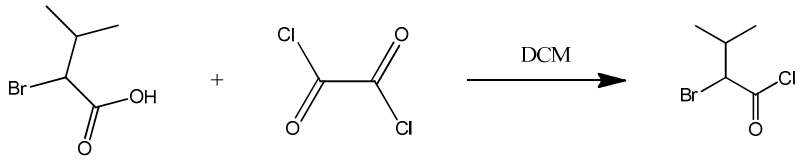


TEA was added as a base. The reaction was monitored by TLC, when the product appeared; reaction mixture was washed with 1M NaOH. Evaporation of the solvent gave product as a yellow solid. Recording to ^{1}H NMR analysis of the product, the obtain product was benzoic 2-(benzoyloxy)-3-methylbutanoic anhydride.

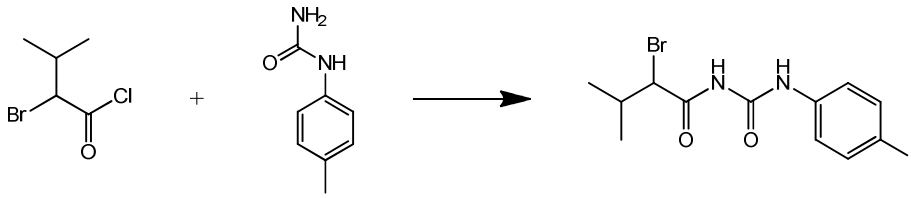
This anhydride was used to synthesize 3-methyl-1-oxo-1-(*p*-tolylamino) butan-2-yl-benzoate, but this reaction didn't work.



In next reaction was syntetized 2-bromo-3-methylbutanoyl chloride at first, which was then treated with p-tolylurea.

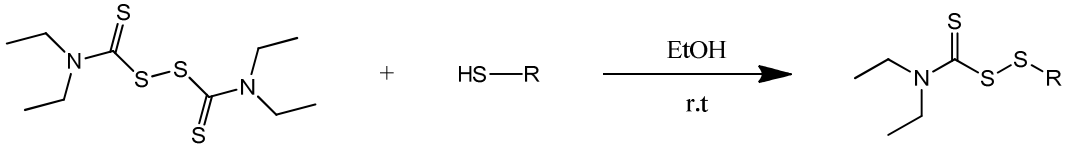


The synthesis of 2-bromo-3-methylbutanoyl chloride was performed in dry conditions two times with yields 64% and 65%. The next reaction with p-tolylurea was performed at 60°C for 2hours, but no product was obtained.



4.3 Synthesis of DPT compounds

For synthesis of DPT compounds was used one step synthesis. A mixture of disulfiram and appropriate thiol was stirred in ethanol at r.t and monitored by TLC.



	R	Molecular Weight	Yield
DPT 001	n-hexyl	265.50	33%, yellow oil
DPT 002	Methylpropan	237.44	4%, yellow oil
DPT 003	Hydroxyethyl	225.40	no product
DPT 004	Cyclohexyl	263.48	8%, yellow oil
DPT 005	i-propyl	223.42	13%, yellow oil
DPT 006	Butyl	237.45	5%, yellow oil
DPT 011	2-pyridyl	258.43	no product
DPT 012	2-methoxybutyl	251.48	38%, yellow oil

The reaction mixture was stirred for 4days until product appeared on TLC. The reaction mixture was purified by column chromatography (96% Hx, 4% EA). Product came as a second spot. Evaporation of solvent gave product as greenish or yellow oil with characteristic smell.

The synthesis of DPT 003 was performed two times in EtOH at r.t, but no new spot appeared. Then this reaction was also performed three times in EtOH at 78°C and purified by column chromatography, but obtained compound wasn´t DPT 003.

In synthesis of DPT 006 two spots came together after column chromatography (96%Hx, 4%EA) so the reaction mixture was purified again by column chromatography (98%Hx, 2%EA).

The synthesis of DPT 011 was monitored by TLC, but no new spot appeared.

^{1}H NMR analysis and mass spec analysis of the products confirmed that the product had been synthesized. Full NMR data, mass spec and full procedure of these compounds are in section 6.3.

Chapter 5

Conclusion

The aim of this study was to synthesise three types of derivatives as part of the investigation undergoing at the Welsh School of Pharmacy to identify potent and selective BCA2- inhibitors without ALDH inhibitory activity characteristic to disulfiram.

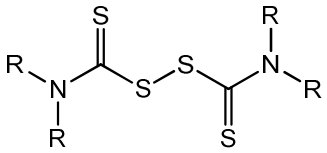
During this investigation was synthesised a big amount of compounds representing different classes of sulphur-based structures (see fig.1.9). A total of 50 compounds were evaluated for antiproliferative activity in the BCA2- positive breast cancer cell lines MCF-7, T47D, MDA-MB-231/ER and the BCA2 – low/negative breast cancer cell line MDA-MB-231 and the normal breast epithelial cell line MCF10A. It was examined at which concentration these compounds would produce 50% grown inhibition (IC_{50}) in tumour cells. Compounds requiring IC50 concentration > 10 μM were considered inactive. The desired compound has to inhibit positive breast cancer cell lines and be inactive against MDA-MB-231 and MCF10A cell line. Results of testing these compounds are summarized in fig.5.1 (21).

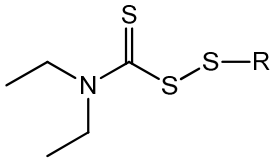
All of the carbamo(dithioperoxo)thioates were active, however only DPT 001 and DPT 003 and were also inactive against MDA-MB-231 and MCF10A cell line. Similarly two disulfiram analogs were active and were considered for further biological testing in comparison to disulfiram. None of tested dithiocarbamates, dithiodimorpholine or benzisothiazolones were active and these results confirmed that an N(C=S) S-S group is required for selective activity in the BCA2 – expressing breast cancer cell lines (21).

The active selective compounds were tested for ALDH inhibition. While DSF and compound 2 inhibited ALDH1 to 57 and 79%, compound 3 and DPT compounds showed little inhibition of this enzyme. Disulfiram analog 3 inhibited only negligible fraction of ALDH1 (3.4%) and is therefore the most selective BCA2 E3 ligase inhibitor amongst the series of disulfides tested in this study. DPT compounds also exhibited low levels of ALDH inhibition (21).

As a result of this study is discovery of selective BCA2 inhibitory antitumor agents that lacked the ALDH – inhibitory activity. Further studies are ongoing to define the antitumor potential of the lead compounds and their associated stability and toxicity.

All these information about biological testing of different potential BCA2 inhibitors as well as description of the chemical synthesis has been published in an article Exploring the structural requirements for inhibition of the ubiquitin E3 ligase breast cancer associated protein 2(BCA2) as a treatment for Breast cancer in Journal of Medicinal Chemistry.





	R
1.Disulfiram	Ethyl
2.	- $(CH_2)_5$
3.	- $(CH_2)_4$

	R		R
4. DPT 001	n-hexyl	**7.** DPT 004	cyklohexyl
5. DPT 002	i-butyl	**8.** DPT 005	i-propyl
6. DPT 003	hydroxyethyl	**9.** DPT 006	n-butyl

Compound	**MCF-7**	**MDA-MB-231**	**MDA-MB-231/ER**	**T47D**	**MCF10A**
1.	0.1± 0.01	>10	0.32 ± 0.14	0.17 ± 0.03	10 ± 0.2
2.	0.03 ± 0.001	>10	0.59 ± 0.12	0.27 ± 0.02	>10
3.	0.35 ± 0.05	3.0 ± 0.4	0.29 ± 0.1	0.33 ± 0.02	>10
4.	0.43 ± 0.1	>10	0.35 ± 0.28	0.23 ± 0.02	>10
5.	0.45 ± 0.25	6.0 ± 2.8	2.1 ± 0.43	0.20 ± 0.02	>10
6.	0.5 ± 0.24	>10	2.75 ± 0.17	0.30 ± 0.03	>10
7.	0.3 ± 0.28	0.96 ± 0.01	0.28 ± 0.17	0.18 ± 0.02	>10
8.	0.39 ± 0.28	0.96 ± 0.03	0.25 ± 0.1	0.23 ± 0.01	>10
9.	0.4 ± 0.3	7.4 ± 2.6	0.3 ± 0.13	0.15 ± 0.02	>10

Fig5.1

Chapter 6

Experimental

6.1 General Procedures

The chemicals used in this investigation were obtained from Sigma-Aldrich and were used without further purification. All glassware were washed and dried before each experiment.

Solvents were evaporated using the Buchi Rotavapor. Melting points were measured on a Griffin apparatus using a capillary method and because of that aren´t exact.

Mass spectra were recorded on a Bruker MicroTOF LC instrument or at the EPSRC National Mass Spectrometry Centre (Swansea, U.K.).

NMR spectra were recorded on a Bruker AVANCE 500 MHz instrument; coupling constants (*J* values) are in Hz. DMSO and $CDCl_3$ were used as solvents.

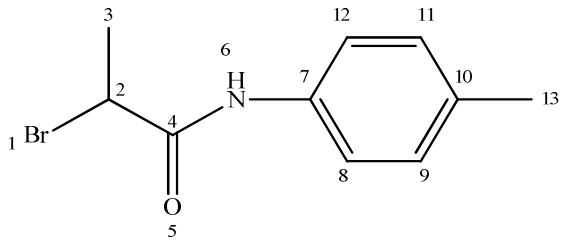
TLC was performed using Merck TLC silica gel 60 plates F_{254} (40-60μM) with detection by UV light (254-366nm).

Purification of compound was achieved via flash chromatography with silica gel 60 μM from Fluka.

6.2 Synthesis of Quinazoline derivatives

6.2.1 QZ 011

6.2.1A 2-bromo-*N*-*p*-tolylpropanamide (Fragment a_1)



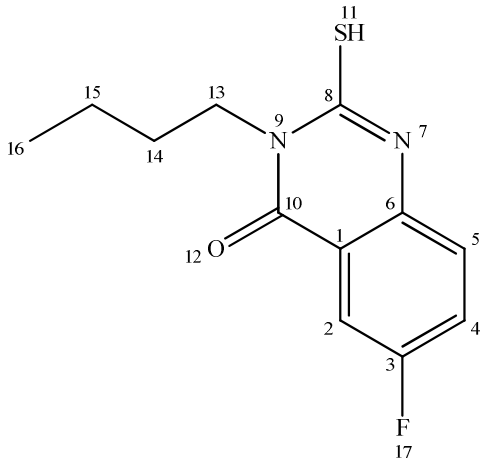
Chemical formula: $C_{10}H_{12}BrNO$

Molecular Weight: 242.11

To a solution of 2-bromopropanoylchloride (0.180ml, 1.75mmol) in DCM (5ml) in round bottom flask was added p-toluidine (0.188g, 1.75mmol). Next, the mixture was stirred at r.t. for 30 minutes while monitored by TLC. Once the starting material disappeared, the solvent was concentrated under vacuum to give the desired product as a powder. Yield 66% (0.296g).

^{1}H NMR ($CDCl_3$) δ 8.00 (1H, s, NH), 7.43 (2H, d, J= 8.7 Hz, C^8-H, C^{12}-H), 7.18 (2H, d, J= 8.3 Hz, C^9-H, C^{11}-H), 4.57 (1H, q, J= 7.2 Hz, C^2), 2.35 (3H, s, C^{13}-H), 1.99 (3H, d, J= 7.1 Hz, C^3-H)

6.2.1B 3-butyl-6-fluoro-2-mercaptoquinazolin-4(3*H*)-one (Fragment b_1)



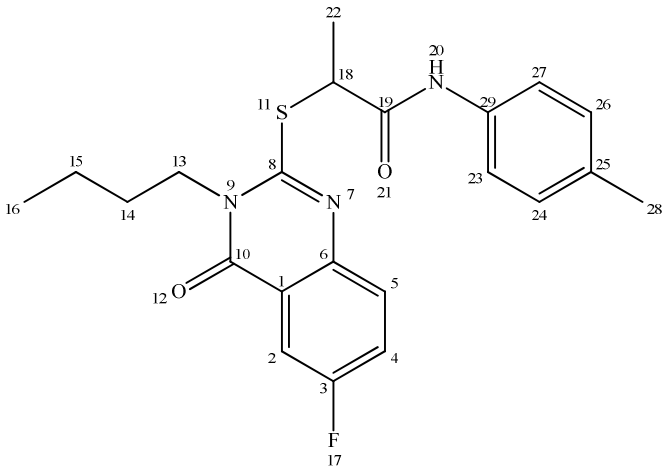
Chemical formula: $C_{10}H_8FN_2OS$

Molecular Weight: 223.25

An equimolar mixture of 2-amino-5-fluorobenzoic acid (0.2g, 1.3mmol) and 1-isothiocyanatobutane (0.15ml, 1.3mmol) in 15 ml of ethanol was stirred continuously at 78°C overnight. Then the reaction turned cloudy with formation of a white precipitate. Further precipitation was induced by cooling the reaction mixture to r.t. The white precipitate was collected by filtration through folded Watman paper and dried in a fume hood. Yield 19% (0.060g).

^{1}H NMR ($CDCl_3$) δ 10.24 (1H, s, SH), 7.82 (1H, dd, J=2.8 Hz, 8.05Hz, C^4-H), 7.41 (1H, m, C^2-H), 7.15(1H, q, J= 4.0 Hz, C^5-H), 4.53 (2H, t, J= 7.9 Hz,C^{13}-H), 1.80 (2H, m, C^{14}-H), 1.48 (2H, m, C^{15}-H), 1.02 (3H, t, J= 7.4 Hz, C^{16}-H)

6.2.1C QZ O11



Chemical Formula: $C_{24}H_{28}FN_3O_2S$

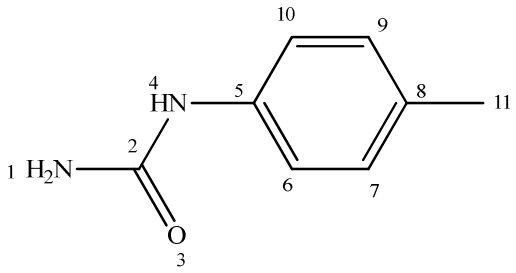
Molecular Weight: 413.51

An equimolar mixture of 2-bromo-*N*-*p*-tolylpropanamide (0.244g, 0.954mmol) and 3-butyl-6-fluoro-2-mercaptoquinazolin-4(3*H*)-one (0.240g, 0.954mmol) and potassium carbonate anhydrous (0.131g, 0.954mmol) in dry DMF was stirred continuously under nitrogen and at r.t. for 36 hours. The reaction was monitored by TLC. When the starting material disappeared, the reaction mixture was poured into a beaker containing 15 ml of ice-water forming a white precipitate which was collected by filtration under vacuum. Yield 52% (0.204g), mp 133°C.

^{1}H NMR ($CDCl_3$) δ 9.71 (1H, s, NH), 7.92 (1H, dd, J=2.9 Hz, 8.4 Hz, C^4-H), 7.66 (1H, q, J=4.7 Hz, C^5-H), 7.53(1H, m, C^2-H), 7.33 (2H, d, J=8.4 Hz, C^{23}-H, C^{27}-H), 7.08 (2H, d, J= 8.4 Hz, C^{24}-H, C^{26}-H), 4.73(1H, q, J= 7.5 Hz, C^{18}-H), 4.13 (2H, t, J = 3.4 Hz, C^{13}-H), 1.77(2H,m, C^{14}-H), 1.71 (3H, d, J = 7.2 Hz, C^{22}-H), 1.59 (3H, s, C^{28}-H), 1.47 (2H,m, C^{15}-H), 1.01 (3H, t, J = 7.2 Hz, C^{16}-H

6.2.2 QZ 012

6.2.2A 1-*p*-tolylurea



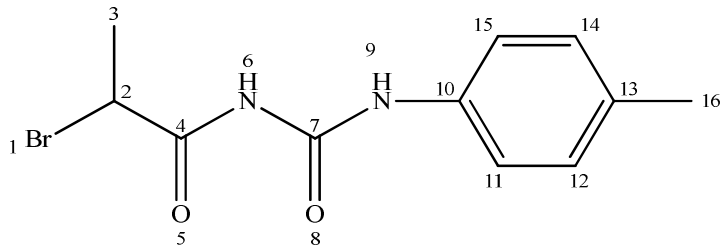
Chemical Formula: $C_8H_{10}N_2O$

Molecular Weight: 150.18

To p-toluidine (0.700g, 6.5mmol) in round bottom flask was added small amount of water, to make a concentrate suspension. To this suspension was added sodium cyanate (0.425g, 6.5mmol) dissolved in 10% acetic acid (10ml) at r.t. The 1-p-tolylurea precipitated as a white solid after 30minutes. The reaction was finished after disappeared all amount of p-toluidine. The precipitate was collected by filtration through folded Watman paper and dried in a fume hood. Yield 25% (0.247g), mp 165°C.

^{1}H NMR ($CDCl_3$) δ 7.45 (2H, s, N^1-H) 7.38 (2H, s, C^6-H, C^{10}-H), 7.28 (2H, s, C^7-H, C^9-H), 6.38 (1H, s, N^3-H), 2.35 (3H, s, C^{11}-H)

6.2.2B 2-bromo-*N*-(*p*-tolylcarbamoyl)propanamide (Fragment a_2)



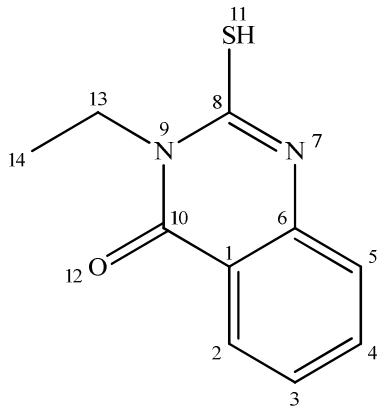
Chemical Formula: $C_{11}H_{13}BrN_2O_2$

Molecular Weight: 285.14

An equimolar mixture of 2-bromopropanoyl chloride (0.14ml, 1.3mmol) and 1-*p*-tolylurea (0.200g, 1.3mmol) without solvent was heated at 60°C for about 1 hour. The reaction generated HCl, which was monitored by pH papers. When it stopped, the reaction mixture was diluted with DCM. Evaporation of the reaction mixture gave yellow solid. Yield 65% (0.246g).

^{1}H NMR ($CDCl_3$) δ 9.97 (1H, s, N^6-H), 8.35 (1H, s, N^9-H), 7.37 (2H, d, J= 8.0 Hz, C^{11}-H, C^{15}-H), 7.12 (2H, m, C^{12}-H, C^{14}-H), 4.56 (1H, q, J=6.6 Hz, C^2-H), 2.32 (3H,d, J=8.4 Hz, C^{16}-H), 1.85 (3H, d, J= 6.8 Hz, C^{13}-H)

6.2.2C 3-ethyl-2-mercaptoquinazolin-4(3*H*)-one (Fragment b_2)

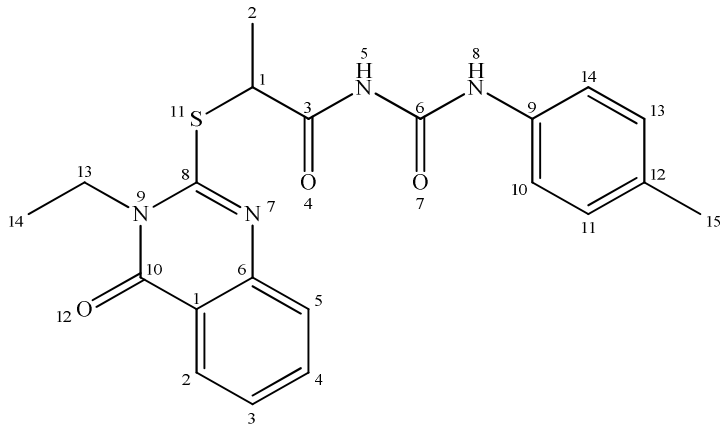


Chemical Formula: $C_{10}H_{12}N_2OS$

Molecular Weight: 208.3

An equimolar mixture of ethylisothiocyanate (1ml, 11.4mmol) and 2-aminobenzoic acid (1.566g, 11.4mmol) in 20ml EtOH was stirred continuously at 78°C overnight. Then the reaction turned cloudy with formation of a white precipitate. Further precipitation was induced by cooling the reaction mixture to r.t. The white precipitate was collected by filtration through folded Watman paper and dried in a fume hood. Yield 57% (1.345g), mp 228°C.

6.2.2D QZ 012



Chemical Formula: $C_{21}H_{22}N_4O_3S$

Molecular Weight: 410.49

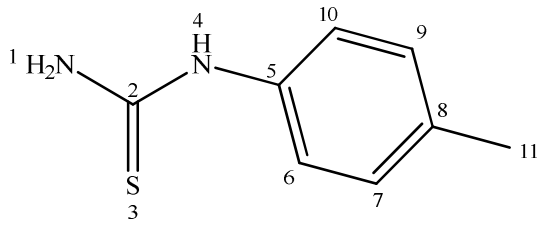
An equimolar mixture of 2-bromo-N-(p-tolylcarbamoyl)propanamide (0.246g,0.86mmol) and 3-ethyl-2-mercaptoquinazolin-4(3H)-one (0.180g,0.86mmol) in dry DMF was stirred in dry condition at r.t. The reaction was monitored by TLC. When the starting material disappeared, the reaction mixture was poured into ice-water forming a precipitate. The white precipitate was collected by filtration through folded Watman paper and dried in a fume hood. Yield 26% (0.093g).

^{1}H NMR ($CDCl_3$) δ 10.61 (1H, s, N^5-H), 10.20 (1H, s, N^8-H), 8.22 (2H,d, J = 8.0 Hz, C^{10}-H, C^{14}-H), 8.11 (2H, d, J= 7.9 Hz, C^{11}-H, C^{13}-H), 7.70 (2H, m, C^2-H, C^5-H), 7.39 (2H, m, C^3-H, C^4-H), 4.65 (1H, q, J= 7.5 Hz, 15.0 Hz, C^1-H), 4.58 (3H, q, J= 7.2 Hz, 14.2 Hz, C^2-H), 2.96 (3H, s, C^{15}-H), 1.69 (3H, d, J= 7.4 Hz, C^{14}-H)

^{13}C NMR ($CDCl_3$) *δ* 13.14, 15.18, 20.79, 40.24, 42.76, 120.17, 124.55, 126.40, 126.59, 127.09, 129.48, 133.87, 134.57, 134.89, 146.44, 150.59, 156.18, 160.83, 173.09

6.2.3 QZ 013

6.2.3A - 1-p-tolylthiourea (Method A)



Chemical Formula: $C_8H_{10}N_2S$

Molecular Weight: 166.2

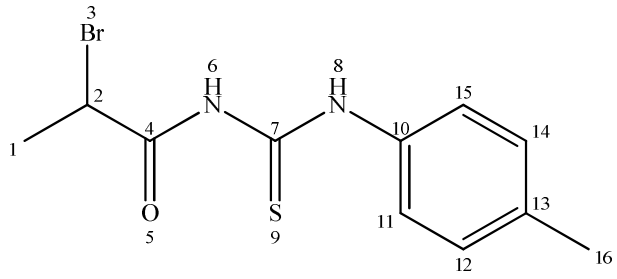
To p-toluidine (0.700g, 6.5mmol) in round bottom flask was added small amount of water, to make a concentrate suspension. To this suspension was added potassium thiocyanate (0.632g, 6.5mmol) dissolved in 10% acetic acid (10ml) at r.t. The reaction was monitored by TLC. After 24h the reaction was stopped. The product was not retrieved.

6.2.3B 1-*p*-tolylthiourea (Method B)

To a stirred solution of *p*-toluidine hydrochloride (1.0g, 6.96mmol) in 15ml THF was added potassium thiocyanate (1.1g, 10.4mmol). The mixture was heated at reflux at 66°C and monitored by TLC. After approximately 3hours TLC analysis indicated the complete consumption of the starting material. The mixture was diluted with 10 ml water and extracted with EA (2x10ml). The organic extracts were combined and washed with HCl (1N, 10ml) and brine (5ml). The organic layer was dried with magnesium sulfate. Evaporation gave product as a yellow solid. Yield 41% (0.478g), mp 92 °C.

^{1}H NMR ($CDCl_3$) δ 8.10 (1H, s, N^4-H), 7.35 (2H, d, J=7.6 Hz, C^6-H, C^{19}-H), 7.10 (2H, d, J=7.7 Hz, C^7-H, C^9-H), 6.15 (1H, s, N^1-H), 2.35 (3H, s, C^{11}-H)

6.2.3C 2-bromo-*N*-(*p*-tolylcarbamothioyl)propanamide (Fragment a_3)



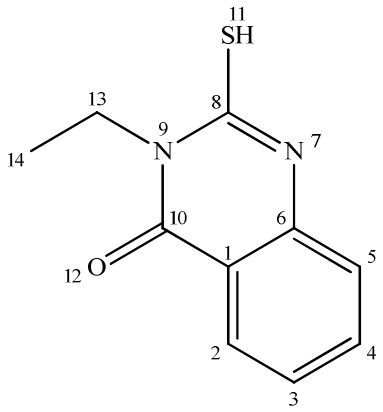
Chemical Formula: $C_{11}H_{13}BrN_2OS$

Molecular Weight: 301.2

An equimolar mixture of 1-*p*-tolylthiourea (0.300g, 1.8mmol) and 2-bromopropanoyl chloride (0.19ml, 1.8mmol) was heated at 60°C. The reaction generated HCl, which was monitored by pH papers. When the reaction stopped to generate HCl, it was finished. The acquired brown liquid was diluted with DCM and EA. Adding EA to this solution induced formation of brown precipitate. Evaporation gave a yellow solid. Yield 44% (0.238g).

^{1}H NMR ($CDCl_3$) δ 12.14 (1H, s, N^6-H), 8.88 (1H, s, N^8-H), 7.42 (2H, d, J=7.8 Hz, C^{11}-H, C^{15}-H), 7.18 (2H, d, J=7.9 Hz, C^{12}-H, C^{14}-H), 4.14(1H, q, J=7.1 Hz, 14.1 Hz, C^2-H), 2.07 (3H, s, C^{16}-H), 1.88 (3H, d, J=7.2 Hz, C^1-H)

6.2.3D 3-ethyl-2-mercaptoquinazolin-4(3*H*)-one (Fragment b_3)

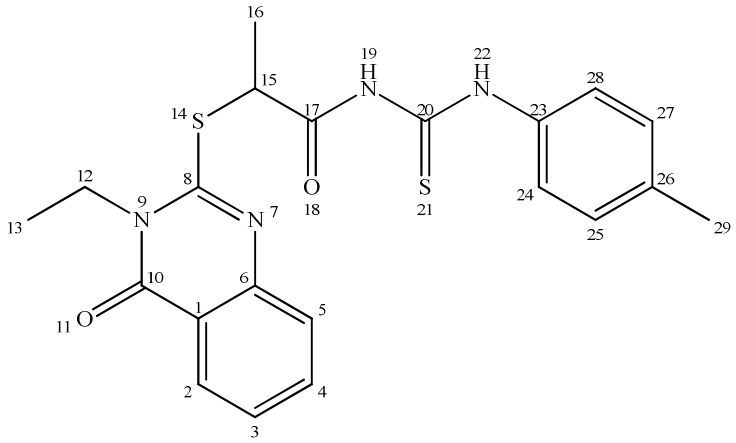


Chemical Formula: $C_{10}H_{12}N_2OS$

Molecular Weight: 208.3

An equimolar mixture of ethylisothiocyanate (1ml, 11.4mmol) and 2-aminobenzoic acid (1.566g, 11.4mmol) in 20ml EtOH was stirred continuously at 78°C overnight. Then the reaction turned cloudy with formation of a white precipitate. Further precipitation was induced by cooling the reaction mixture to r.t. The white precipitate was collected by filtration through folded Watman paper and dried in a fume hood. Yield 57% (1.345g), mp 228°C.

6.2.3E QZ 013



Chemical Formula: $C_{21}H_{22}N_4O_2S_2$

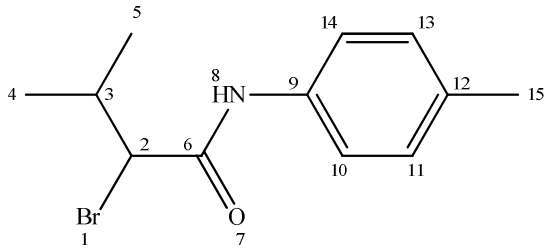
Molecular Weight: 426.55

An equimolar mixture of 2-bromo-N-(p-tolylcarbamothioyl)propanamide (0.200g, 0.66mmol) and 2-ethyl-3-mercaptonaphthalen-1(2H)-one (0.138g, 0.66mmol) in dry DMF (10ml) was stirred at r.t. The reaction was monitored by TLC. After 3 days was only starting material in the reaction mixture. The product was not retrieved.

6.3 Synthesis of derivatives of 2-amino-2-oxoethyl-benzoate

6.3.1 JS 001

6.3.1A - 2-bromo-3-methyl-N-p-tolylbutanamide (Fragment a- Method A)



Chemical Formula: $C_{12}H_{16}BrNO$

Molecular Weight: 270.17

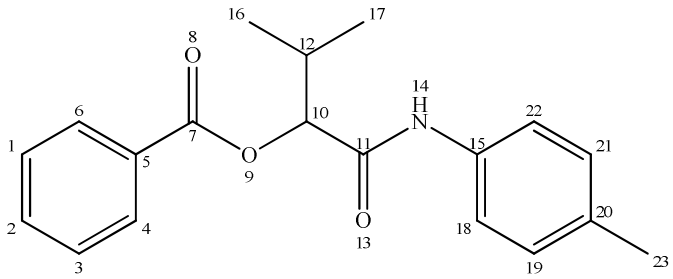
A mixture of 2-bromo-3-methylbutanoic acid (0.300g, 1.7mmol) and EDCI (0.411g, 2.1mmol) in anhydrous DMF (15ml) was stirred continuously under nitrogen at r.t. for 30 minutes. To this solution was added dropwise REA (2.67ml) and p-toluidine (0.182g, 1.7mmol) dissolved in anhydrous DMF. The mixture was stirred overnight and monitored by TLC. Evaporation gave the residue, which was dissolved in EA, washed with 1 N HCl and dried with magnesium sulphate. Evaporation gave a powder, but very little yield.

6.3.1B 2-bromo-3-methyl-*N*-*p*-tolylbutanamide (Fragment a – Method B)

A mixture of 2-bromo-3-methylbutanoic acid (0.344g, 1.9mmol) and p-toluidine (0.204g, 1.9mmol) in DMF (5ml) were treated with EDCI (0.730g, 3.8mmol) and DMAP (0.582g, 4.7mmol). Resulting solution was stirred for 3hours monitored by TLC. When the starting material disappeared the reaction mixture was poured into EA (75ml), washed with 10% aqueous HCl (2x50ml) and 10% aqueous sodium carbonate (3x50ml). The organic phase was dried with magnesium sulfate. Evaporation of solvent gave yellow solid. Yield 50% (0.256g).

^{1}H NMR ($CDCl_3$) δ 8.19 (1H, s, NH), 7.43 (2H, d, J=8.6 Hz, C^{10}-H, C^{14}-H), 7.17 (2H, d, J=8.6 Hz, C^{11}-H, C^{13}-H), 4.44 (1H, d, J= 4.7 Hz, C^2-H), 2.50 (1H, m, C^3-H), 2.35 (3H, s, C^{15}-H), 1.13 (3H,d, J= 6.7 Hz, C^4-H), 1.07 (3H, d, J=6.5 Hz, C^5-H)

6.3.1C methyl-1-oxo-1-(*p*-tolylamino)butan-2-yl-benzoate – JS 001

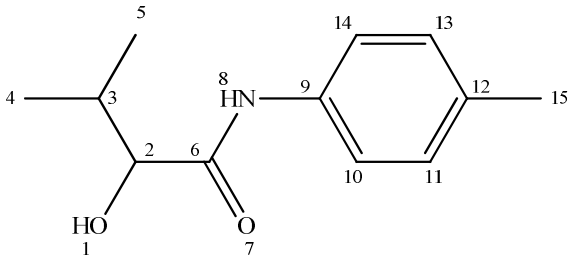


Chemical Formula: $C_{19}H_{21}NO_3$

Molecular Weight: 311.37

A mixture of benzoic acid (0.116g, 0.95mmol) and sodium hydride (0.034g, 1.42mmol) in dry DMF (5ml) was stirred continuously under nitrogen at r.t for 30 minutes. Than was added 2-bromo-3-methyl-N-p-tolylbutanamide (0.256g, 0.95mmol). The mixture was stirred overnight and monitored by TLC. After 24 hours the reaction mixture was poured into ice-water forming a precipitate. The yellow precipitate was collected by filtration through folded Watman paper and dried in a fume hood. Recording to NMR the obtained precipitate was starting material 2-bromo-3-methyl-*N*-*p*-tolylbutanamide.

6.3.1D 2-hydroxy-3-methyl-*N*-*p*-tolylbutanamide



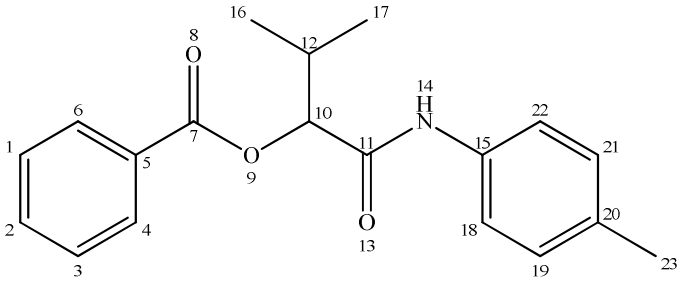
Chemical Formula: $C_{12}H_{17}NO_2$

Molecular Weight: 207.27

A mixture of D- α- hydroxyisovaleric acid (0.150g, 1.27mmol) and p-toluidine (0.136g, 1.27mmol) in DMF (5ml) were treated with EDCI (0.487g, 2.54mmol) and DMAP (0.383g, 3.09mmol). Resulting solution was stirred for 3hours monitored by TLC. When the starting material disappeared the reaction mixture was poured into EA (75ml), washed with 10% aqueous HCl (2x50ml) and 10% aqueous sodium carbonate (3x50ml). The organic phase was dried with magnesium sulfate. Evaporation of solvent gave yellow solid. Yield 33% (0.088g).

^{1}H NMR ($CDCl_3$) δ 8.59 (1H, m, OH), 7.45 (2H, d, J= 8.4 Hz, C^{10}-H, C^{14}-H), 7.12 (2H, d, J=8.2 Hz, C^{11}-H, C^{13}-H), 4.08 (1H,d, J=3.2 Hz, C^2-H), 2.31 (3H,s, C^{15}-H), 1.30 (1H,m, C^3-H), 1.06 (3H,d, J= 6.9 Hz,C^4-H), 0.91 (3H,d,J=6.9 Hz, C^5-H)

6.3.1E methyl-1-oxo-1-(p-tolylamino)butan-2-yl-benzoate – JS 001



Chemical Formula: $C_{19}H_{21}NO_3$

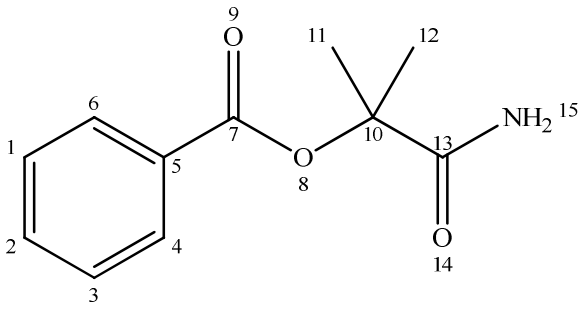
Molecular Weight: 311.37

2-hydroxy-3-methyl-*N*-*p*-tolylbutanamide (0.088g, 0.4mmol) was dissolved in dry DCM (5ml), than added benzoic acid (0.046ml, 0.4mmol) and 1.1 equivalent of TEA(0.065ml, 0.47mmol) and left it stirred at r.t. The reaction was monitored by TLC and finished after 12hours. The reaction mixture was purified by column chromatography. Yield 10% (0.012g).

^{1}H NMR ($CDCl_3$) δ 8.16 (2H, d, J= 7.2 Hz , C^4-H, C^6-H), 7.68 (1H, m, C^2-H), 7.55 (2H, t, J= 7.8 Hz, C^1-H, C^3-H), 7.40 (2H, d, J= 8.31, C^{18}-H, C^{22}-H), 7.14 (2H, d, J= 8.3 Hz, C^{19}-H, C^{21}-H), 5.46 (1H, d, J= 4.5 Hz, C^{10}-H), 4.25 (1H, m, C^{12}-H), 2.33 (3H, s, C^{23}-H), 1.15 (3H,d, J= 6.8 Hz, C^{17}-H), 1.11 (3H, d, J= 7.0 Hz, C^{17}-H)

6.3.2 JS 002

6.3.2A – 1-amino-2-methyl-1-oxopropan-2-yl-benzoate



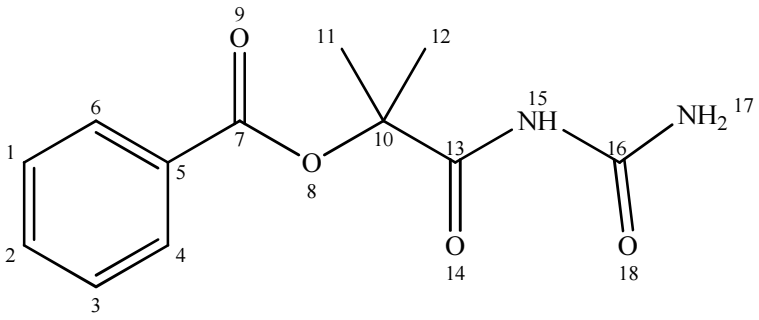
Chemical Formula: $C_{11}H_{13}NO_3$

Molecular Weight: 207.23

A mixture of benzoic acid (0.292g, 2.4 mmol) and 2-bromo-2-methylpropanamide (0.200g, 1.2mmol) in acetonitrile in water (6ml/0.3ml) was stirred at r.t. Then silver oxide was added in one part. The reaction was stirred at r.t for 2 hours. The reaction mixture was filtered through Celite and washed with EA. Evaporation of the filtrate gave a powder product. Yield 71% (0.177g).

^{1}H NMR ($CDCl_3$) δ 8.04 (2H, d, J= 7.7 Hz, C^4-H, C^6-H), 7.61 (1H, t, J= 7.6 Hz, C^2-H), 7.48 (2H, t, J= 7.7 Hz, C^1-H, C^3-H), 6.66 (1H,s, C^{10}-H), 1.79 (3H,s), 1.49 (3H, s)

6.3.2B 2-methyl-1-oxo-1-ureidopropan-2-yl-benzoate – JS 002



Chemical Formula: $C_{12}H_{14}N_2O_4$

Molecular Weight: 250.25

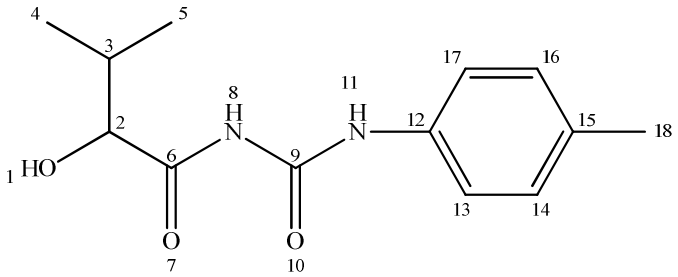
1-amino-2-methyl-1-oxopropan-2-yl benzoate (0.177g, 0.8mmol) was dissolved in dioxane (108ml) and treated with 1-chloro-2-isocyanatoethane (1.1ml, 6mmol). The reaction was stirred for 3 hours at r.t. Then added methanol (8.6ml) and Zn dust (2.16g) and left it stirred for next 3hours. Zn dust was removed by filtration through Celite. Evaporation gave yellow solid. Yield 448% (0.896g). The product was sent for mass spec analysis, which confirmed that the obtained product isn't JS 002.

^{1}H NMR (DMSO) δ 7.97 (1H, d, J= 7.3 Hz), 7.59 (2H, m), 7.35 (2H, m), 1.59 (3H, s), 1.23 (3H, s)

m/z (AP$^+$CI) 391.32 [M$^+$+H]

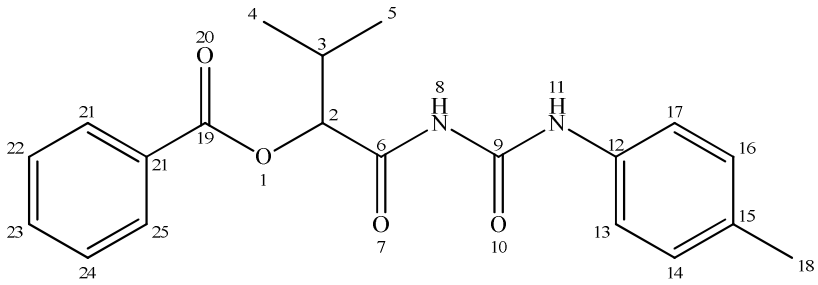
6.3.3 JS 003

6.3.3A 2-hydroxy-3-methyl-N-(p-tolylcarbamoyl)butanamide



A mixture of D- α- hydroxyisovaleric acid (0.150g, 1.27mmol) and p-tolylurea (0.191g, 1.27mmol) in DMF (5ml) were treated with EDCI (0.487g, 2.54mmol) and DMAP (0.383g, 3.09mmol). Resulting solution was stirred for 3hours monitored by TLC. When the starting material disappeared the reaction mixture was poured into EA (75ml), washed with 10% aqueous HCl (2x50ml) and 10% aqueous sodium carbonate (3x50ml). The organic phase was dried with magnesium sulfate. Evaporation of solvent gave yellow solid. Yield 11% (0.041g).

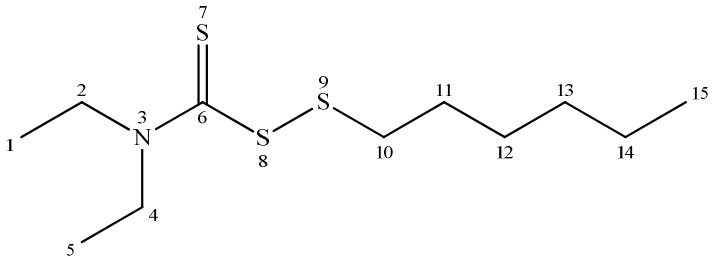
6.3.3B 3-methyl-1-oxo-1-(3-*p*-tolylureido)butan-2-yl-benzoate – JS 003



An equimolar mixture of 2-hydroxy-3-methyl-N-(p-tolylcarbamoyl)butanamide (0.041g, 0.139mmol) and benzoyl chloride (0.016ml, 0.139mmol) in dry DCM (5ml) was stirred at room temperature. TEA (0.02ml, 0.139mmol) was added as a base. The reaction was monitored by TLC. Product wasn´t retrieved.

6.4 Synthesis of DPT compounds

6.4.1 DPT 001 - hexyl diethylcarbamo(dithioperoxo)thioate



Chemical Formula: $C_{11}H_{23}NS_3$

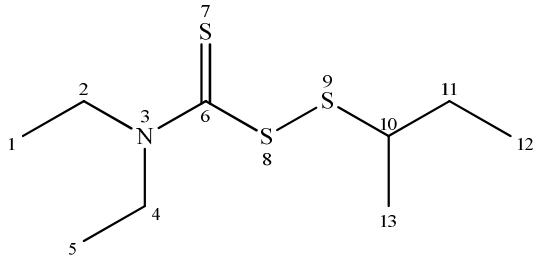
Molecular Weight: 265.50

A mixture of disulfiram (0.351g, 1.2mmol) and 1.5 equivalent of hexane-1-thiol (0.25ml, 1.8mmol) in ethanol (15ml) was stirred at r.t. for 3 days. The reaction mixture was purified by column chromatography (96% Hx, 4% EA). The obtained product was yellow oil. Yield 33% (0.154g).

^{1}H NMR ($CDCl_3$) δ 4.05 (2H, s), 3.82(2H, s), 2.86(2H, t, J=7.5 Hz), 1.66(2H, m), 1.41(2H, m), 1.31(10H, m), 0.89(3H, m)

m/z (AP^+CI) 266.11 [M^++H]

6.4.2 DPT 002 - *iso*butyl diethylcarbamo(dithioperoxo)thioate



Chemical Formula: $C_9H_{19}NS_3$

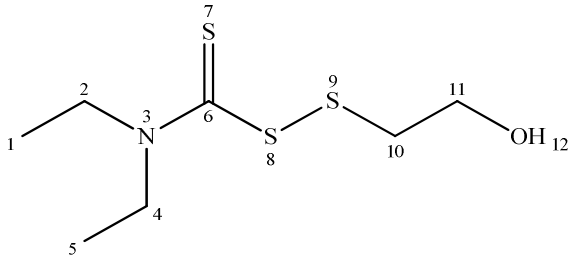
Molecular Weight: 237.44

A mixture of disulfiram (1.360g, 4.6mmol) and 2 equivalents of butane-2-thiol (1ml, 9.2mmol) in ethanol (15ml) was stirred at r.t. for 4 days. The reaction mixture was purified by column chromatography (96% Hx, 4% EA). The obtained product was yellow oil. Yield 4% (0.045g).

^{1}H NMR ($CDCl_3$) δ 4.07(2H, s), 3.87(2H, s), 3.12(1H, m, C^{10}-H), 1.77(1H, m), 1.56(1H, m), 1.32(9H, m), 1.02(3H, m)

m/z (AP^+CI) 238.09 [M^++H]

6.4.3 DPT 003 - 2-hydroxyethyl diethylcarbamo(dithioperoxo)thioate

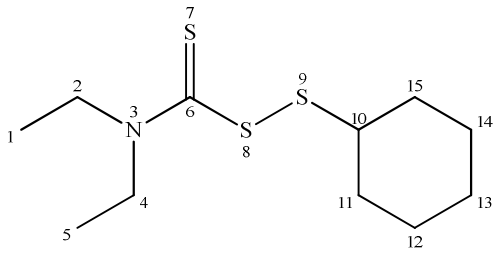


Chemical Formula: $C_7H_{15}NOS_3$

Molecular Weight: 225.40

An equimolar mixture of disulfiram (4.23g, 1.4mmol) and mercaptoethanol (1ml, 1.4mmol) in ethanol (15ml) was stirred at r.t for four days. The reaction mixture was purified by column chromatography (90% Hx, 10% EA). The product was not retrieved.

6.4.4 DPT 004 – cyclohexyl diethylcarbamo(dithioperoxo)thioate



Chemical Formula: $C_{11}H_{21}NS_3$

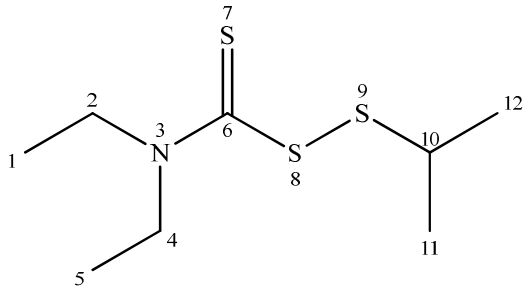
Molecular Weight: 263.48

A mixture of disulfiram (1.22g, 4.1mmol) and 2 equivalents of cyclohexanethiol (1ml, 8.2mmol) in ethanol (15ml) was stirred at r.t. for 4 days. The reaction mixture was purified by column chromatography (96% Hx, 4% EA). The obtained product was yellow oil. Yield 8% (0.085g).

^{1}H NMR ($CDCl_3$) δ 4.07(2H, s), 3.86(2H, s), 2.07(2H, s), 1.80(2H, s), 1.62(1H, s), 1.33(12H, m)

m/z (AP^+CI) 264.09 [M^++H]

6.4.5 DPT 005 – isopropyl diethylcarbamo(dithioperoxo)thioate



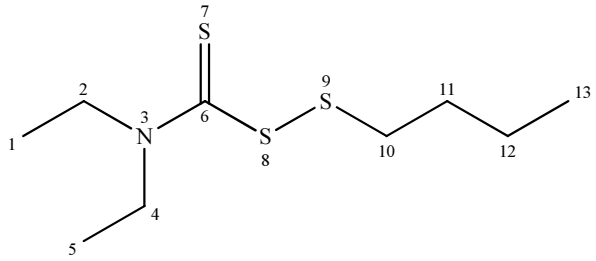
Chemical Formula: $C_8H_{17}NS_2$

Molecular Weight: 223.42

A mixture of disulfiram (1.59g, 5.4 mmol) and 2 equivalents of isopropylthiol (1ml, 10.7 mmol) in ethanol (15ml) was stirred at r.t. for 4 days. The reaction mixture was purified by column chromatography (96% Hx, 4% EA). The obtained product was yellow oil. Yield 13% (0.162g).

^{1}H NMR ($CDCl_3$) δ 4.08(2H, s), 3.87(2H, s), 3.31(1H, s), 1.35(9H, m), 0.94(3H, m)

6.4.6 DPT 006 - butyl diethylcarbamo(dithioperoxo)thioate



Chemical Formula: $C_9H_{19}NS_3$

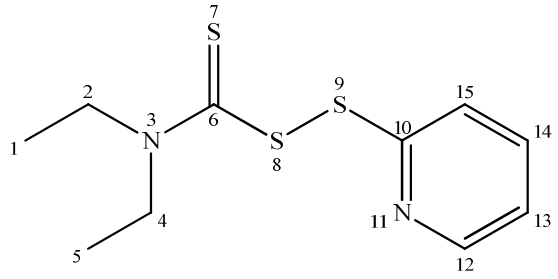
Molecular Weight: 237.45

A mixture of disulfiram (1.38g, 4.7mmol) and 2 equivalents of butanethiol (1ml, 9.3mmol) in ethanol (15ml) was stirred at r.t. for 4 days. The reaction mixture was purified by column chromatography (98% Hx, 2% EA). Yield 5%.

^{1}H NMR ($CDCl_3$) δ 4.06(2H, s), 3.83(2H, s), 2.87(2H, t, J=7.4 Hz, C^{10}-H), 1.66(2H, m), 1.44(2H, m), 1.33(6H, s), 0.93(3H, t, J=7.3 Hz)

m/z (AP^+CI) 238.07 [M^++H]

6.4.7 DPT 011 – pyridin-2-yl diethylcarbamo(dithioperoxo)thioate

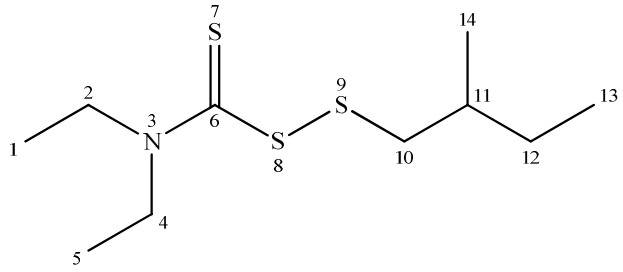


Chemical Formula: $C_{10}H_{14}N_2S_3$

Molecular Weight: 258.43

A mixture of disulfiram (0.435g, 1.5mmol) and 2 equivalents of mercaptopyridine (0.326, 2.9mmol) in ethanol (15ml) was stirred at r.t. for 4 days. The reaction mixture was purified by column chromatography (96% Hx, 4% EA). The product was not retrieved.

6.4.8 DPT 012 - 2-methylbutyl diethylcarbamo(dithioperoxo)thioate



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A mixture of disulfiram (1.206g, 4.1mmol) and 2 equivalents of 2-methylbutane thiol (1ml, 8.1mmol) in ethanol (15ml) was stirred at r.t. for 4 days. The reaction mixture was purified by column chromatography (96% Hx, 4% EA). Yield 38%.

^{1}H NMR ($CDCl_3$) δ 4.07(2H, s), 3.83(2H, s), 2.92(1H, m), 2.73(1H,m), 1.34(9H, s), 1.04(3H, d, J=6.7 Hz), 0.92(3H, t, J=7.5Hz)

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List of figures

Fig 1.1 adapted from UK breast Cancer incidence statistics. *Cancer Research UK.* [Online] 2009. [Cited: August 1, 2009]. http://info.cancerresearchuk.org/cancerstats/types/breast/incidence/index.htm.

Fig 1.2 adapted from **Pickart, CM.** Mechanisms underlying ubiquitination. *Annu Rev Biochem.* 2001, vol. 70, 503-533.

Fig 1.3 adapted from **Hershko, A.; Ciechanover, A.** The ubiquitin system. *Annu Rev Biochem.* 1998, vol. 67, 425-479.

Fig 1.4 adapted from **Lecker, SH.; Goldberg, AL.; Mitch, WE.** Protein degradation by the ubiquitin-proteasome pathway in normal and disease states. *J Am Soc Nephrol.* 2006, vol. 17, 1807-1819.

Fig 1.5 adapted from **Peter Kosarev, Klaus FX Mayer, and Christian S Hardtke**. Evaluation and classification of RING-finger domains encoded by the Arabidopsis genome. *Genome Biol.* 2002, vol. 3, issue 4, research 0016.

Fig 1.6 adapted from **Pickart, CM.** Mechanisms underlying ubiquitination. *Annu Rev Biochem.* 2001, vol. 70, 503-533.

Fig 1.7 adapted from **Weissman, AM.** Themes and variations on ubiquitylation. *Mol Cell Biol.* 2001, vol. 2, issue 3, 169-178.

Fig 1.8 adapted from **Burger, A.;Amemiya, Y.; Kitching, R. et al.** Novel RING E3 ubiquitin ligases in breast cancer. *Neoplasia.* 2006, vol. 8, issue 8, 689-695.

Fig 5.1 adapted from **Brahemi, G; Kona, F; Fiasella A. et al.** Exploring the stuctural requirements for inhibition of the ubiquitin E3 ligase breast cancer associated protein 2 (BCA2) as a treatment for breast cancer. *J. Med. Chem.* 2010, vol. 53(7), 2757-2765.

Appendix

a.) QZ 011

b.) QZ 012

c.) JS 001

d.) DPT 001

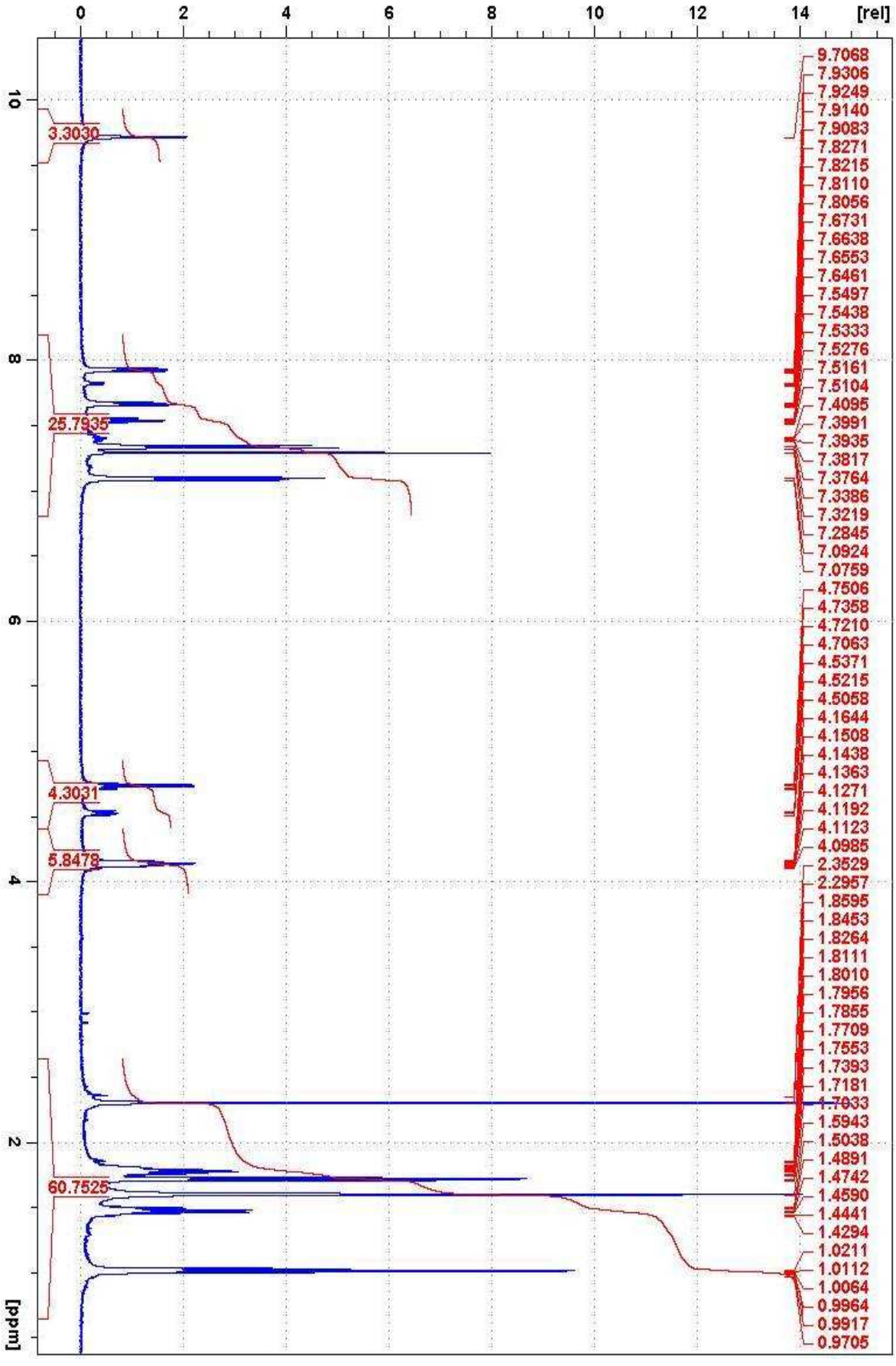
e.) DPT 002

f.) DPT 004

g.) DPT 005

h.) DPT 006

i.) DPT 011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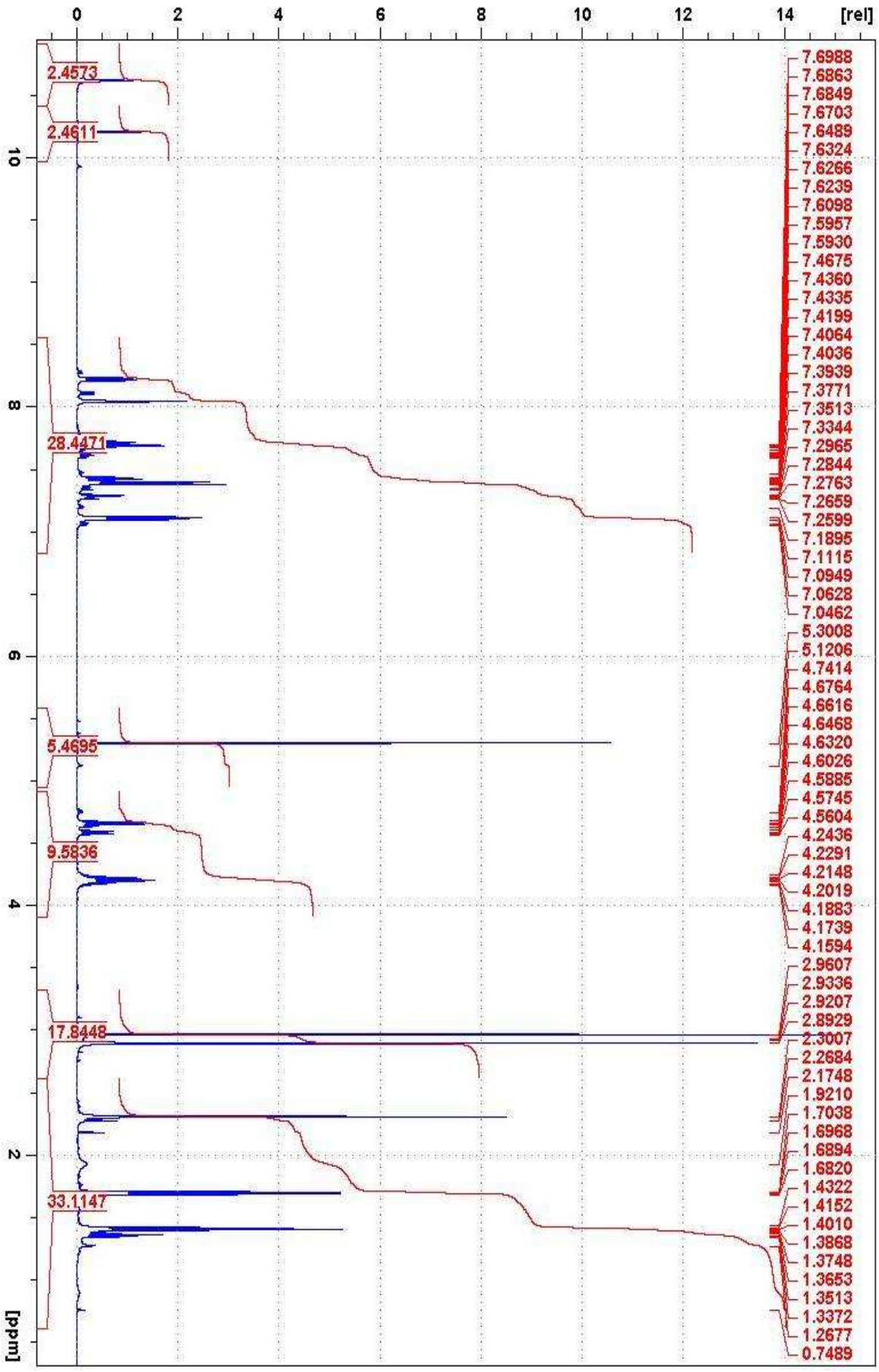
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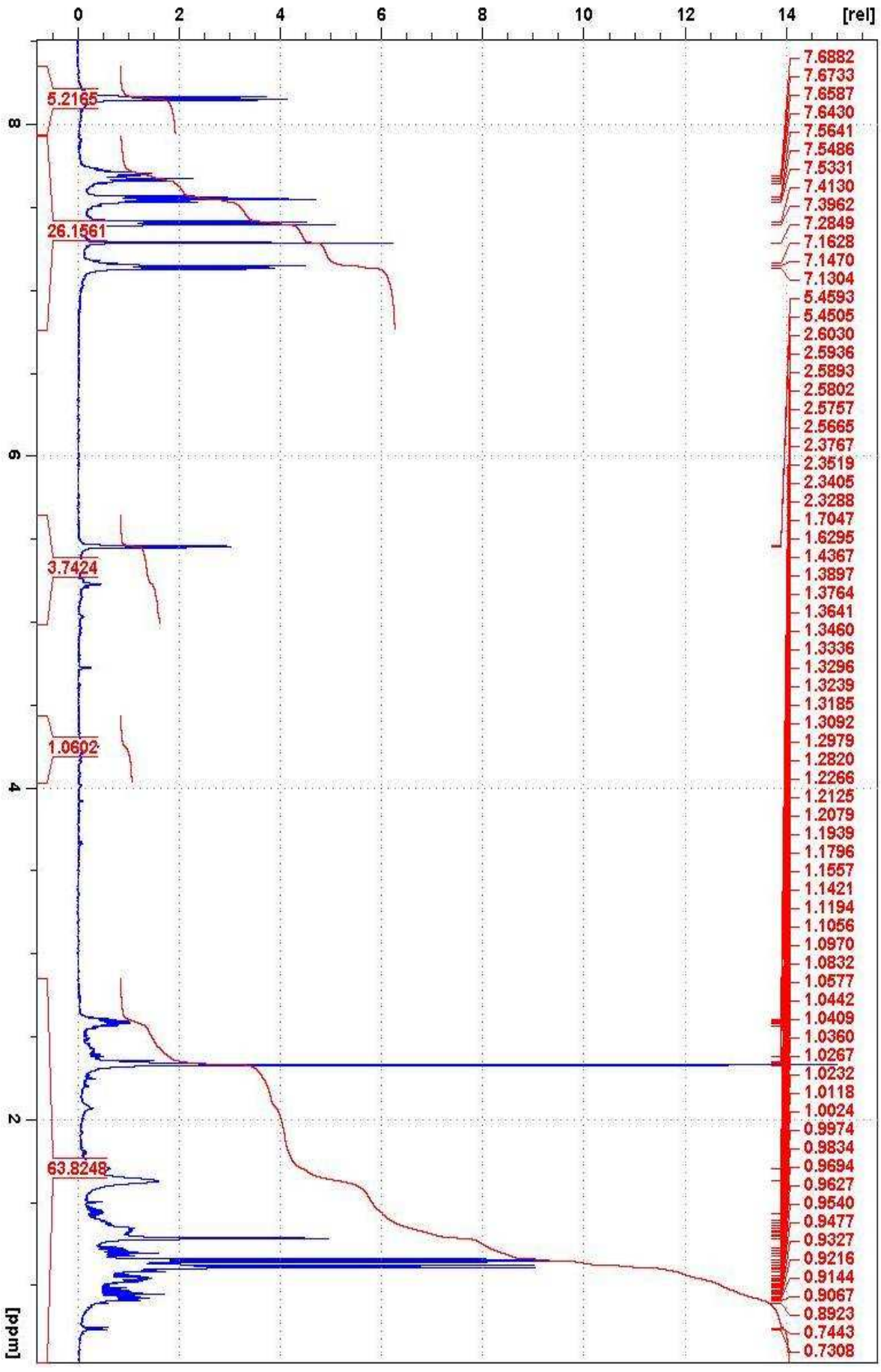
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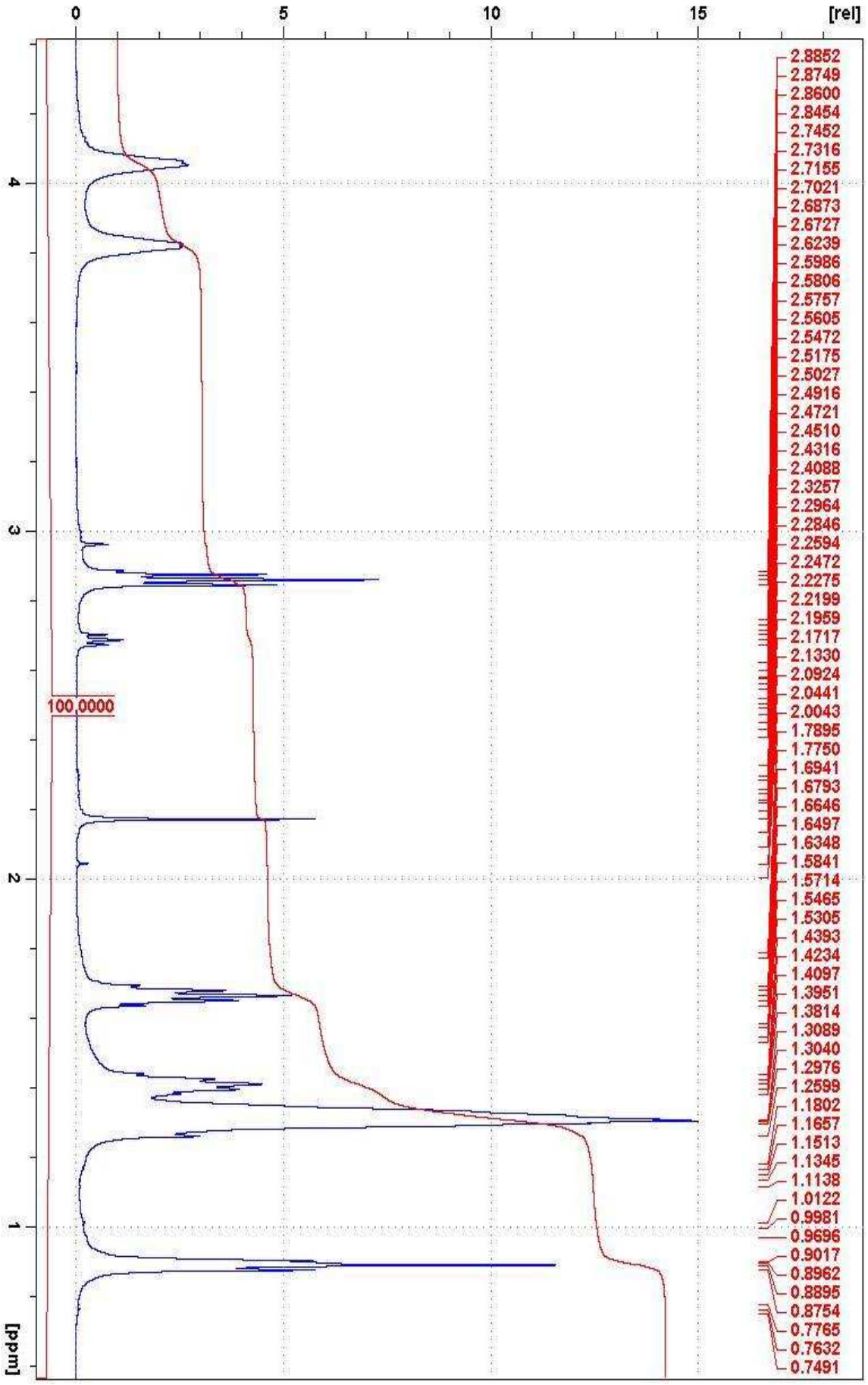
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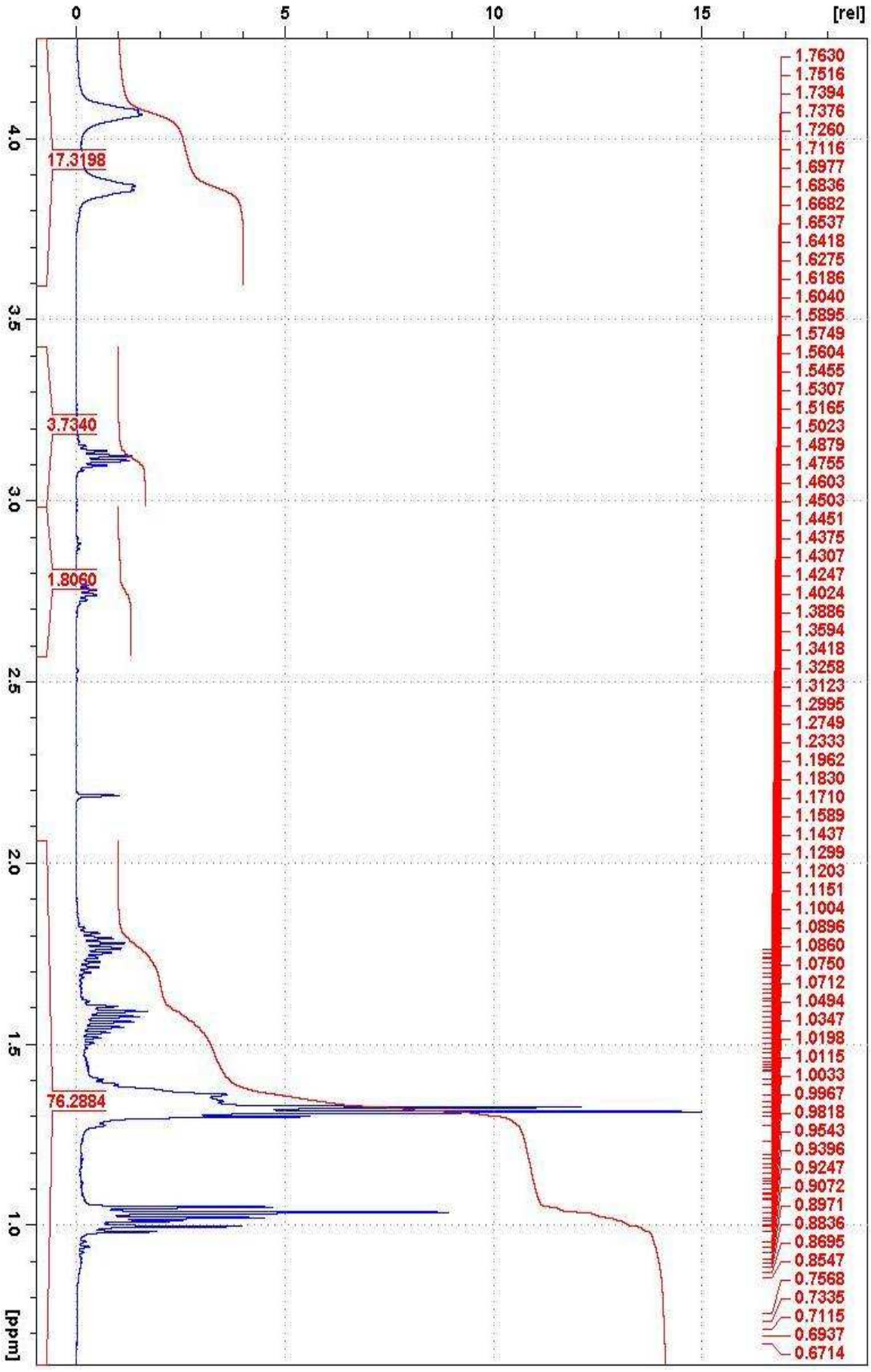
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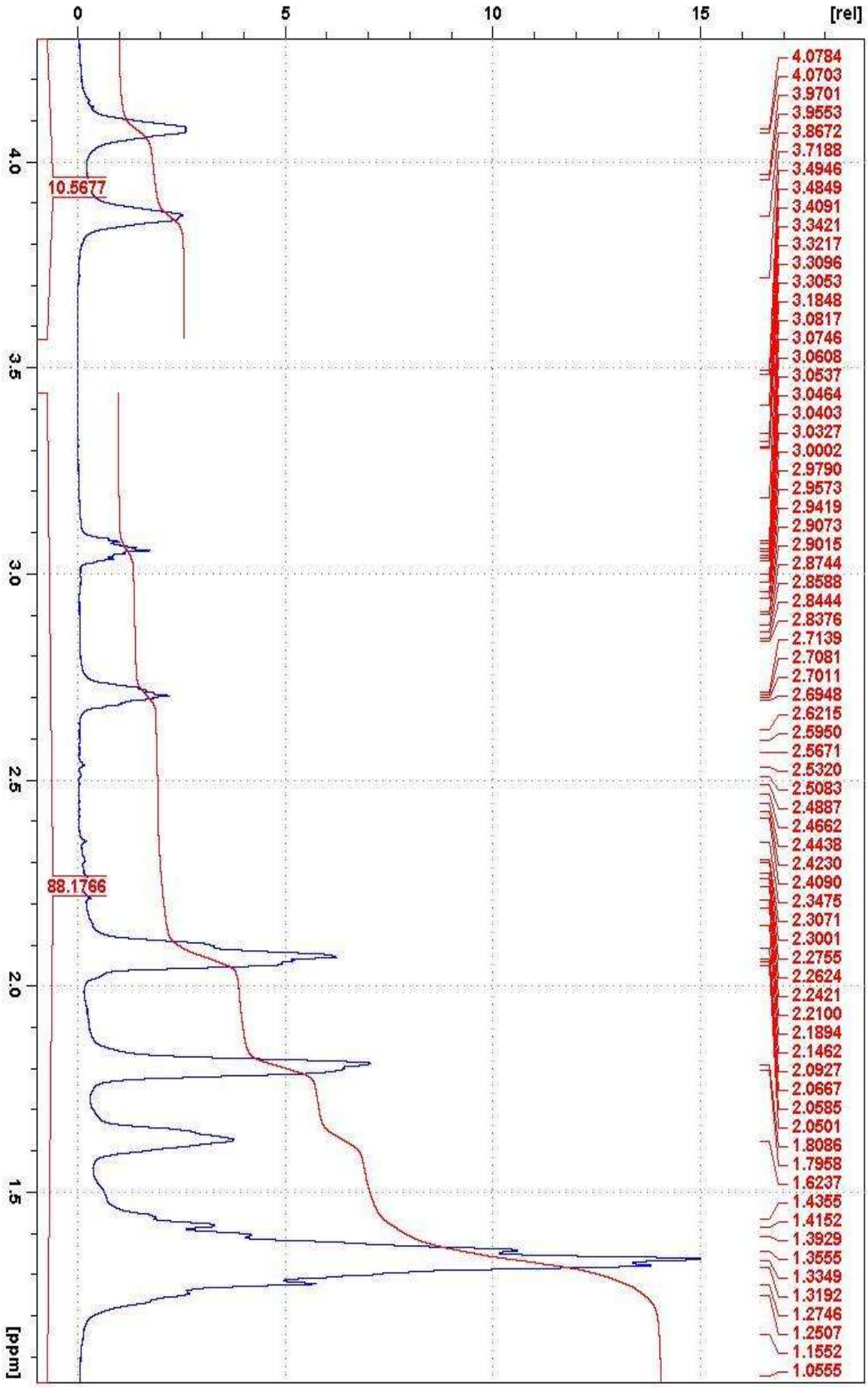
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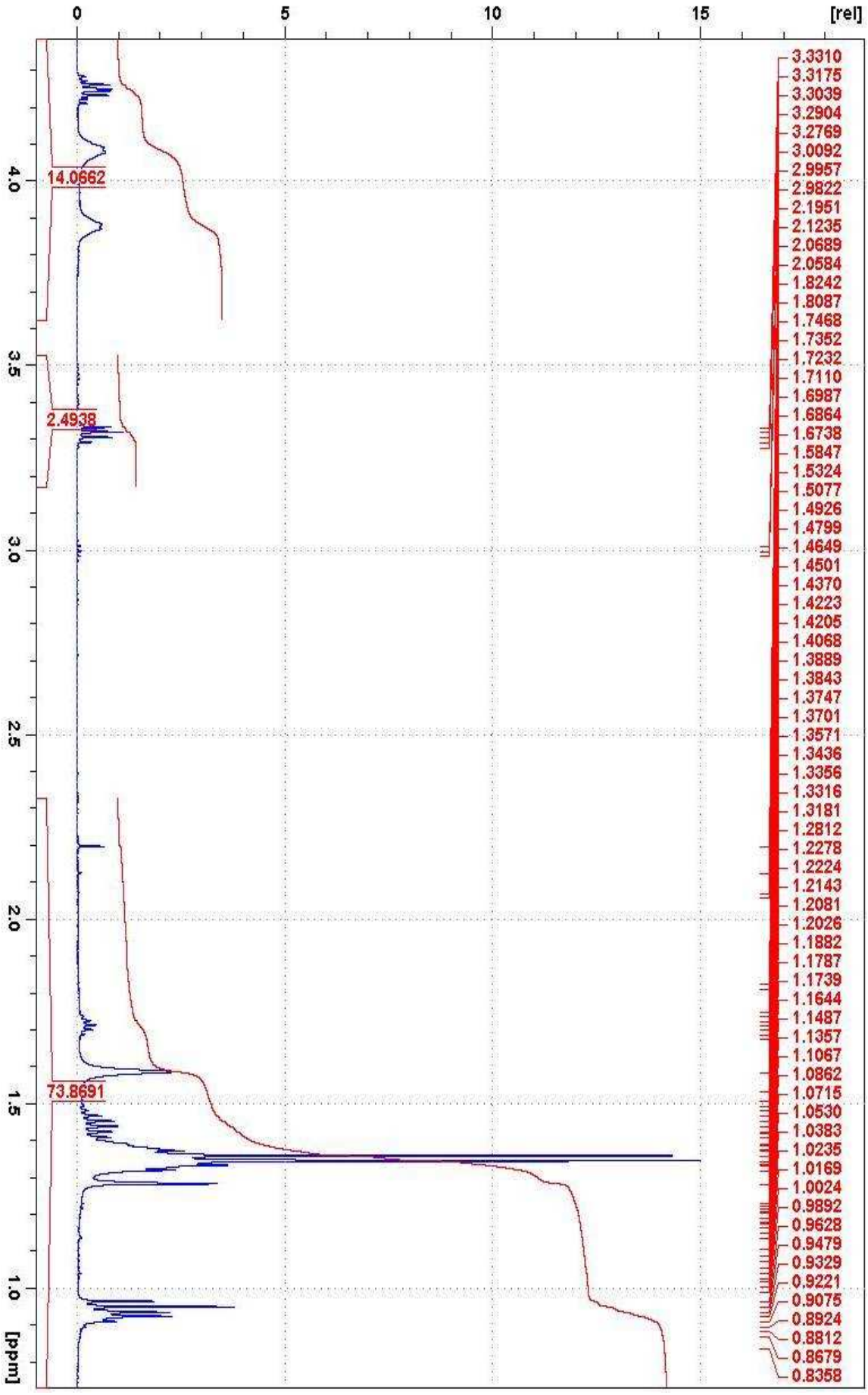
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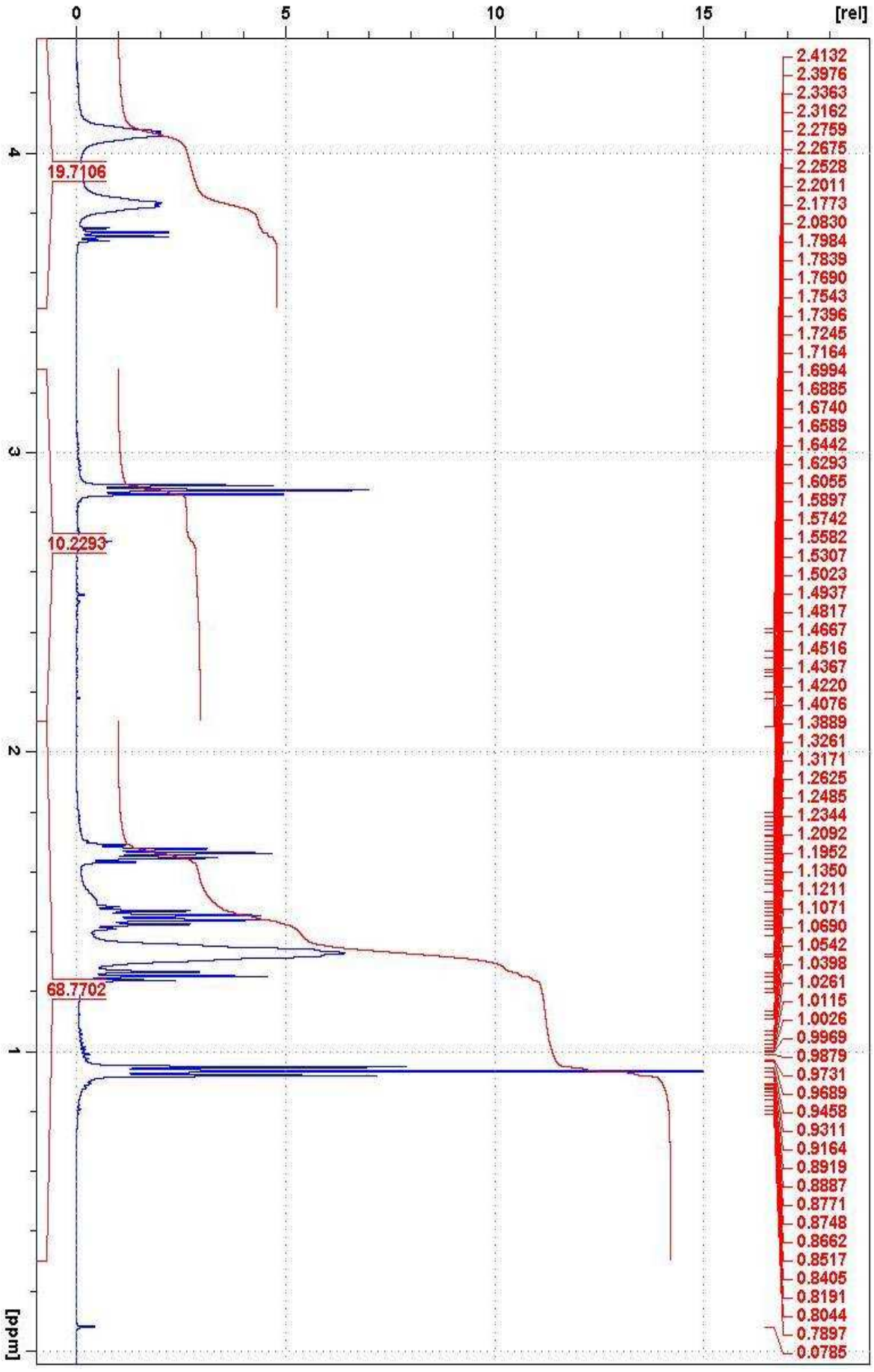
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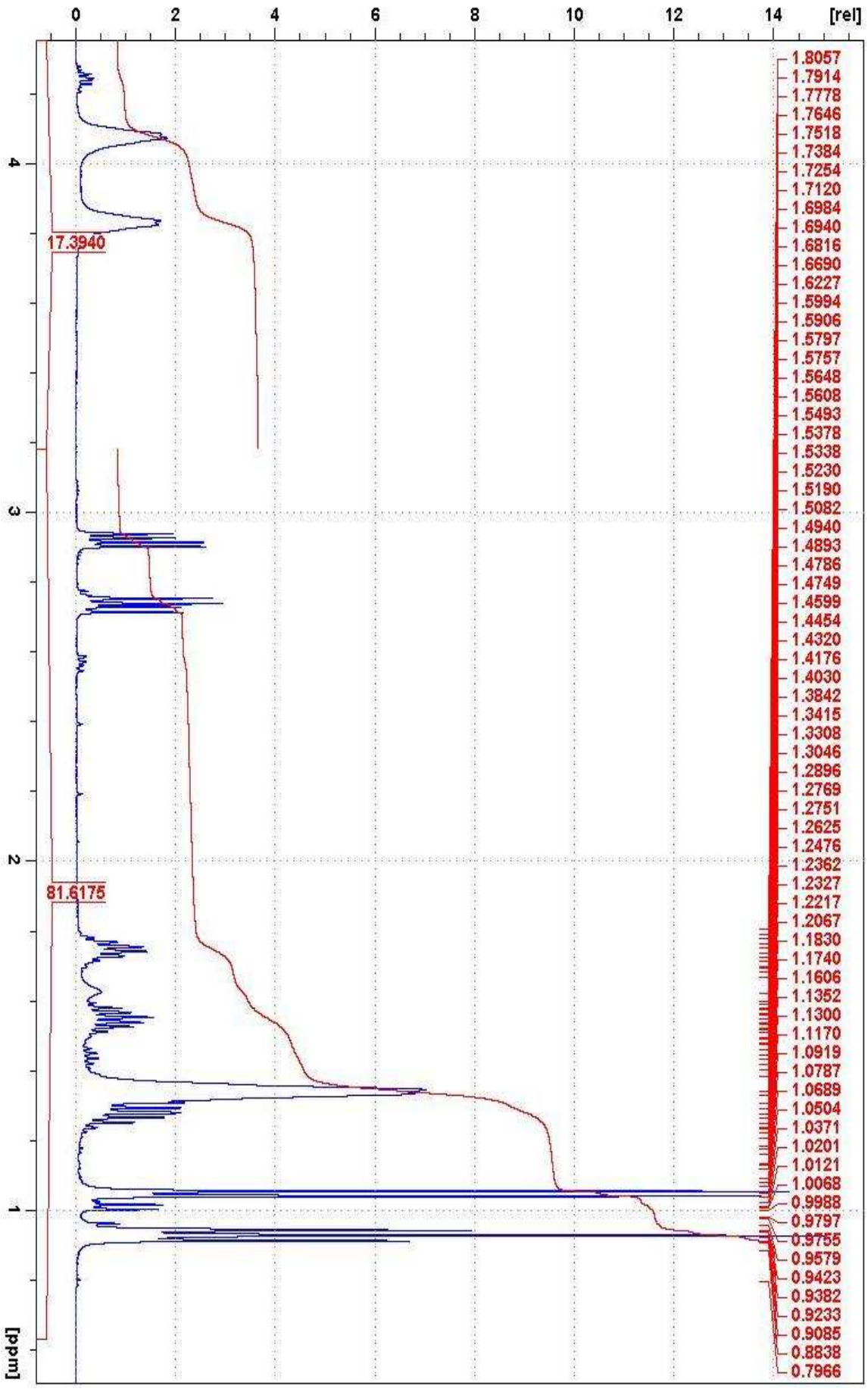
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Evaluation

Evaluation of the four months internship of Jitka Soukupová at Cardiff University

Jitka Soukupová worked in the Welsh School of Pharmacy, Cardiff University during the period May – September 2009, on a project concerned with the design and synthesis of potential inhibitors of BCA2, an E3 ubiquitin ligase enzyme implicated in breast cancer development.

Jitka worked alongside a PhD student in the Welsh School of Pharmacy (Ghali Brahemi), carrying out the laboratory synthesis of new drug candidates for testing as BCA2-inhibitory drug candidates by our US collaborators. I found Jitka to be a hard-working and intelligent student, with the enthusiasm and motivation to achieve good results in her laboratory studies where she is technically proficient.

She has learned a great deal in the areas of drug design, synthesis and the process of cancer drug discovery during her studies, and I found her theoretical knowledge to be good. She was a popular and friendly member of the research group in Cardiff with good communication skills, and always reliable and trustworthy. She is capable of independent work, however works well with other people also. Jitka has enjoyed her visit to the UK, and has made many friends from within the School of Pharmacy and Cardiff University.

Jitka has successfully synthesized a number of exciting drug candidates for testing against breast cancer cells, and completed a number of synthetic targets and project objectives that contribute to a very good and successful work placement. She has proved to be one of the best Erasmus students that we have had here in Cardiff.

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