CHARLES UNIVERSITY IN PRAGUE FACULTY OF PHARMACY IN HRADEC KRÁLOVÉ

Department of Pharmaceutical Chemistry and Drug Control

Synthetic routes to 2-phenylbenzothiazoles with potential application in cancer therapy and PET imaging

Diploma Thesis

Supervisors:

Assoc. Prof. RNDr. Veronika Opletalová, Ph.D.

Dr. Andrew Westwell Ph.D.

Hradec Králové, 2013 Jan Frydrych

„This thesis is the result of my own independent work/investigation, except where otherwise stated. Other sources are acknowledged by explicit references. This work has not previously been accepted in substance for any degree and is not concurrently submitted in candidature for any degrees“.

Signed:…… **Date:…...…..**

Acknowledgements

I would like to express my sincere gratitude to my supervisors,Dr. Andrew Westwell and Assoc. Prof. Veronika Opletalová without whose expertise and leading this thesis would never have existed.

I also must acknowledge all the staff and students at the Welsh School of Pharmacy who helped me throughout this work.

I want to thank the Erasmus programme for my Welsh School of Pharmacy internship where all laboratory work was made.

This work was supported by SVV 267001/2013 programme.

Abstract

There are three main tasks reported in this thesis. The first is the report of an improved procedure for synthesis of biologically relevant 2-phenylbenzothiazoles with various substituents on phenyl ring. Reported 2-phenylbenzothiazoles were synthesised by heating equimolar amounts of 2-aminothiophenol disulfides with appropriate benzaldehydes with *p*-toluenesulfonic acid in the presence of polymer-bound triphenylphosphine using mixture of toluene and DMF as a solvent. Main features of reported method include simple product isolation (removal polymer-bound by-product by filtration through Celite® layer), avoidance of column chromatography, rapid synthesis and good yields of correspondent benzothiazole.

The second goal of this thesis is the solution phase synthesis of 2-phenylbenzothiazoles bearing different substituents on both the benzothiazole and phenyl ring. Attempts were made to synthesise different 2-phenylbenzothiazoles by heating equimolar amount of substituted 2-aminobenzothiazoles with relevant benzaldehydes in high-boiling solvents using sodium metabisulfite as mild oxidant. The results of this method were unconvincing. We got several traces of desired compound with 6-methyl or 6-methoxy substituted 2-aminobenzothiazoles but in other cases we could not isolate our desired compounds.

The third task was the synthesis of precursors of [^{18}F]-radiolabelled 6-fluoro-2-(2,3-dimethoxyphenyl)benzothiazole and 5-fluoro-2-(2,3-dimethoxyphenyl)benzothiazole (GW 610) as potential PET agents for Alzheimer´s disease diagnosis and developing potential methods for their radiolabelling. Particularly of interest was the synthesis of [^{18}F] fluorinated GW 610 because of its extraordinary anticancer activity *in vitro* and *in vivo*, reported in recent years. Successful synthesis of 2-(2,3-dimethoxyphenyl)-6-nitrobenzothiazole via Jacobson cyclisation and the direct aromatic nucleophilic substitution of the nitro precursor using F^- in presence of Kryptofix 2.2.2® using both DMSO and DMF as a solvent to establish condition for future radiolabelling was subsequently performed. Furthermore two candidates for [^{18}F]-F labelling, namely 2-(2,3-dimethoxyphenyl)-6-tributylstannylbenzothiazole and 2-(2,3-dimethoxyphenyl)-5-tributylstannylbenzothiazole (GW610) were synthesised via Jacobson cyclisation followed by palladium-catalyzed stannylation. Both organotin compounds can be used

for both direct [^{18}F] fluorination using [^{18}F]-F, and for more favourable preparation even more reactive diaryliodonium salt, suitable precursors for [^{18}F]$^{-}$ / Kryptofix 2.2.2® labelling.

Abstrakt

Tato práce pojednává o třech hlavních úkolech. Za prvé zde bude referováno o nové pokročilé syntetické metodě vedoucí k biologicky aktivním 2-fenylbenzothiazolům, substituovaných na benzenovém kruhu různými substituenty. Tyto 2-fenylbenzothiazoly byly syntetizovány zahříváním ekvimolárního množství 2-aminothiofenoldisulfidu s vhodnými benzaldehydy za přítomnosti *p*-toluensulfonové kyseliny a na polymerní nosič navázaného trifenylfosfinu. Jako rozpouštědlo byla zvolena směs toluenu a DMF. Jednoduchá izolace produktu (oddělení na polymer navázaného vedlejšího produktu reakce filtrací přes vrstvu Celitu®), díky čemuž není nutná izolace produktu pomocí sloupcové chromatografie, dobré výtěžky reakcí a rychlá syntéza jsou největší výhody této metody.

Druhým úkolem byla syntéza 2-fenylbenzothiazolů nesoucích různou substituci jak na benzenovém, tak na benzothiazolovém kruhu. Byly provedeny experimenty se zahříváním ekvimolárních množství substituovaných 2-aminobenzothiazolů s vhodnými aldehydy ve vysokovroucích rozpouštědlech za použití disiřičitanu sodného jako mírného oxidačního činidla. Výsledky této metody byly neuspokojivé. Ačkoli v případě 6-methyl a 6-methoxy substituovaných 2-aminobenzothiazolů byly nalezeny stopy produktu, v ostatních případech jsme produkt nebyli schopni izolovat.

Posledním úkolem byla syntéza prekurzorů [^{18}F]-značených 6-fluoro-2-(2,3-dimethoxyfenyl)benzothiazolu a 5-fluoro-2-(2,3-dimehoxyfenyl)benzothiazolu (GW 610) jako potenciálních ligandů pro PET s možným využitím pro diagnostiku Alzheimerovy choroby, a vyvinout metodiku pro jejich radioaktivní značení. Objektem zájmu je zejména syntéza [^{18}F]-značeného GW 610, neboť v dřívějších studiích vykázal protirakovinnou aktivitu a to jak *in vitro,* tak *in vivo*. Jacobsonovou cyklizací byl syntetizován 2-(2,3-dimethoxyfenyl)-6-nitrobenzothiazol, s nímž byla následně provedena přímá nukleofilní substituce na aromatické jádro s použitím aniontu F^- jako nukleofilu v přítomnosti Kryptofixu 2.2.2 s užitím DMSO nebo DMF jako rozpouštědla. Tato reakce byla provedena za účelem stanovení optimálních podmínek pro potenciální budoucí značení radioaktivním fluoridovým aniontem. Dále byly pomocí Jacobsonovy cyklizace následované paladiem-katalyzovanou stanylací, syntetizovány dvě sloučeniny potenciálně využitelné pro značení [^{18}F]-F jmenovitě

2-(2,3-dimethoxyfenyl)-6-tributylstanyl-benzothiazol a 2-(2,3-dimethoxyfenyl)-5-tributylstanyl-benzothiazol. Obě organocínové sloučeniny mohou být značeny buď s použitím radioaktivního fluoru ([^{18}F]-F), nebo pro syntézu reaktivnějších diaryliodoniových solí, vhodných prekurzorů pro značení aniontem fluoru [^{18}F]$^{-}$ v přítomnosti Kryptofixu 2.2.2

Content:

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Abbreviations

°C	**-**	**degree Celsius**
%	**-**	**Percentage**
[^{18}F]-FDG	**-**	**[^{18}F]-labelled 2-fluoro-2-deoxy-D-glucose**
5F 203	**-**	**2-(4-amino-3-methylphenyl)-1,3-benzothiazol-6-ol**
A549	**-**	**adenocarcinoma human alveolar basal epithelial cells**
AD	**-**	**Alzheimer´s disease**
AhR	**-**	**arylhydrocarbon receptor**
ALS	**-**	**amyotrophic lateral sclerosis**
Ar	**-**	**aryl**
Aβ	**-**	**β-amyloid**
BBB	**-**	**blood–brain barrier**
BTA	**-**	**benzothiazole aniline**
CJM 126	**-**	**(4-aminophenyl)-1,3-benzothiazol**
CYP 1A1	**-**	**cytochrome P450, family 1, subfamily A, polypeptide 1**
DAIB	**-**	**[di(acetoxy)iodo]-benzene**
DCM	**-**	**dichloromethane**
DF 203	**-**	**(4-amino-3-methylphenyl)-1,3-benzothiazol**
DME	**-**	**dimethoxyethane**
DMSO	**-**	**dimethylsulfoxide**
DNA	**-**	**deoxyribonucleic acid**
DS	**-**	**Down´s syndrome**
EtOH	**-**	**ethanol**
FBTA	**-**	**[^{18}F] 2-[4-(2-fluoroethyl)aminophenyl]-6-hydroxybenzothiazole**

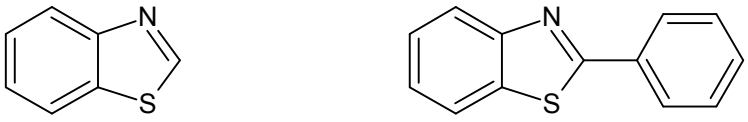
GI_{50}	**-**	**50% growth inhibition starting from time zero**
HCC 2998	**-**	**colon cancer cell line**
HCT-116	**-**	**colon cancer cell line**
HL-60	**-**	**leukaemia cell line**
HPLC	**-**	**high-performance liquid chromatography**
HT29	**-**	**colon cancer cell line**
IC_{50}	**-**	**inhibition constant 50%**
IGROV1	**-**	**human ovarian cancer cell line**
Ki	**-**	**affinity constant of the displacer compound for the receptor**
KM12	**-**	**colon cancer cell line**
LR	**-**	**Lawesson´s reagent**
MAMA	**-**	**monoamine-monoamide**
MCF-7	**-**	**human breast cancer cell line**
MDA 468	**-**	**human breast cancer cell line**
MeOH	**-**	**methanol**
***n*-BuOH**	**-**	***n*-butanol**
NCL- H322M	**-**	**human lung cancer cell line**
NMP	**-**	***N*-Methyl-2-pyrrolidone**
NMR	**-**	**nuclear magnetic resonance**
Pd(PPh3)4	**-**	**tetrakis(triphenylphosphine)palladium(0)**
$Pd_2(dba)_3$	**-**	**tris(dibenzylideneacetone)dipalladium**
PDAIS	**-**	**poly[4-diacetoxyiodo]styrene**
PEPPSI	**-**	**pyridine, enhanced, precatalyst, preparation, stabilisation and initiation**
PET	**-**	**positron emission tomography**

PiA	**-**	**Pittsburgh compound A**
PiB	**-**	**Pittsburgh compound B**
PPh_3	**-**	**triphenylphosphine**
***p*TSA**	**-**	***p*-toluenesulfonic acid**
rt	**-**	**room temperature**
SNU-638	**-**	**gastric carcinoma cell line**
SPECT	**-**	**single photon emission computed tomography**
SR	**-**	**leukaemia cell line**
TBAB	**-**	**tetrabutylammonium bicarbonate**
TES	**-**	**triethylsilane**
TFA	**-**	**trifluoroacetic acid**
ThT	**-**	**Thioflavin T**
TLC	**-**	**thin layer chromatography**
TOPO I	**-**	**topoisomerase I**
TOPO II	**-**	**topoisomerase II**
TZDM	**-**	**2-[4-(dimethylamino)phenyl]-6-iodobenzothiazole**

Chapter 1

1.1 Introduction and the Aim of the Work

Benzothiazole, **1,** have shown a wide range of biological activity in recent years. It played a role as an important pharmacophore in medicinal chemistry, and compounds bearing benzothiazole structure in their molecule posses a number of both *in vitro* and *in vivo* pharmacologic effects. This makes the benzothiazoles potential treatments in a number of both therapeutic and diagnostic settings. As will be shown below some of benzothiazole compounds have been proven to be safe for human treatment and are currently used as a treatment for several diseases. In this thesis I will focus on 2-arylbenzothiazoles,**2,**.Once their pharmacological properties and applicability in medicinal field has been demonstrated, the synthetic approaches in recent years will be overviewed and related problems will be discussed. I will focus on 2-arylbenzothiazoles and related compounds with potential therapeutic potential in Alzheimer´s disease diagnosis and cancer treatment. In practical part of this thesis several new methods leading to substituted 2-phenylbenzothiazoles will be shown and their advantages and shortcomings will be discussed. Furthermore my second task, synthesis of [^{18}F]labelled 2-phenylbenzothiazoles as potential positron emission tomography agent, will be displayed and the results discussed.



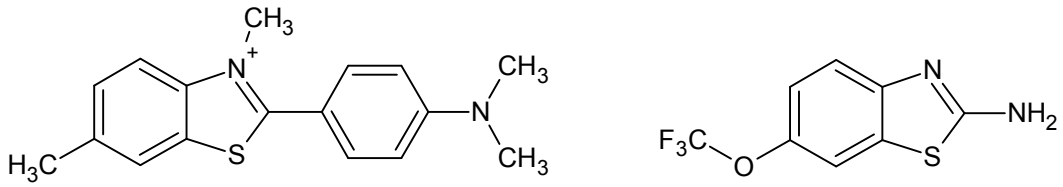
1 **2**

Figure 1.Benzothiazole and 2-phenylbenzothiazole

1.2. Benzothiazoles currently used in medicine

Thioflavin T (ThT, **3**) is an organic cationic dye invented in 1959. ThT hascurrently been used for amyloid fibrils visualisation both *in vivo* and *in vitro*. Amyloid is a pathologic and insoluble protein which is associated with many diseases such as Alzheimer´s disease (AD), Huntington disease, Type 2 diabetes and many more. ThT possesses enhanced fluorescence and a characteristic shift (480nm, when excited at 450 nm) in its emission spectrum upon binding to amyloid protein. Because of this feature ThT is used for amyloid detection in tissue using fluorescence microscopy and amyloid fibril growth using total internal reflection fluorescence microscopy.[1, 2]

The next benzothiazole structure bearing compound currently used in medicine is riluzole,**4**, Riluzole inhibits the releasing of glutamate in presynaptic nerve endings by blocking tetrodotoxine sensitive sodium channel [3]. It is indicated to extend live time or extend time without mechanical ventilation for patients with amyotrophic lateral sclerosis (ALS) .Riluzole has been proved by clinical trials to extend survival for patient with ALS. It is marketed by Sanofi-Aventis with the brand nameRilutek[4]. In the Czech Republic, riluzole is available as the preparation Riluzol PMCS in 50mg dose.



3 **4**

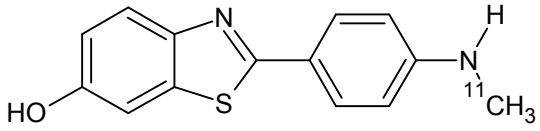
Figure 2.Thioflavin T and riluzole

1.3. Benzothiazole as non-invasive agent for Alzheimer´s disease diagnosis

Nowadays, one of the most valuate medicinal application of substituted 2-phenylbenzothiazoles is the non-invasive diagnosis of Alzheimer´s disease. As shown further, some of them are currently being tried in clinical evaluation stages.

Dementia represents a number of disorders which all have in common the loss of cognitive ability. AD is the most frequent form of dementia affecting in the United Kingdom more than 800000 people and in the Czech Republic 14 923 people were treated with the diagnosis F00 (dementia associated with AD) in 2011.In both countries the numbers are increasing every year [5,6]. AD was firstly demonstrated by Dr.Alois Alzheimer in 1907. It is a neurodegenerative disorder characteristic with memory loss and learning disturbance. For AD characteristic hallmark is the presence of extracellular β-amyloid (Aβ) plaques and intracellular filamentous tangles of polyphosphorylated protein tau. Current clinical diagnosis of AD is mainly based on cognitive tests.The cognitive disorders however appear in progressive stage of the disease which seems to be the main drawback of cognitive tests. Aβ plaques however have been found in early stage of AD and therefore there is the interest to visualise them before the disease progress and begin the treatment immediately. The most research in scope of Aβ visualisation is based on positron emission tomography (PET) and single photon emission computed tomography (SPECT). Both of these methods involve the introduction of short-live radionucleotides into small molecule with affinity to Aβ. However, PET has better resolution and higher sensitivity in comparison with SPECT. Therefore it is used more than SPECT 7.

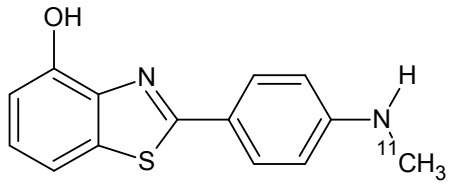
2-arylbenzothiazoles have shown great ability to show Aβ plaques in human brain. The highest activity was shown in group of [^{11}C]-labelled 6-substituted 2-(4-aminophenyl)benzothiazole. These compounds have shown to cross the brain-blood barrier, high Aβ binding potential and fast elimination from intact brain tissue 8. Definitely the most promising compound of [^{11}C]-labelled 6-substituted 2-(4-aminophenyl)benzothiazole group was the PiB,5, [^{11}C]-labelled 6-hydroxy- 2-(4-*N*-methylaminophenyl)benzothiazole. This compound is based on, **4,**and it became the first compound based on 2-arylbenzothiazole structure to enter clinical evaluation as potential AD diagnostic agent. It also displayed a large retention in several parts of brain [9]. The hydroxyl group was introduced in previously synthesised compound known as PiA to enhance brain clearance of unbound compound and therefore make the results more accurate [10].



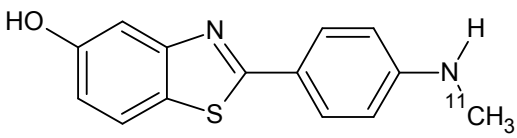
5

Figure 3. PiB

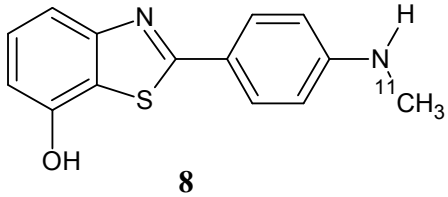
Because of high promises of PiB, several new ^{11}C labelled analogues of this compound were synthesised. In 2009, K. Serdon´s group developed three labelled isomers of PiB, 4-hydroxy-2-(4´-[^{11}C]methylamino)-1,3-phenylbenzothiazole ,**6**, 5-hydroxy-2-(4´-[^{11}C]methylamino)-1,3-phenylbenzothiazole ,**7**, and7-hydroxy-2-(40-[^{11}C]methylamino)-1,3-phenylbenzothiazole,**8,**. Compounds ,**7,** and ,**8,** showed almost the same log P as PiB (2,48 ; 2,45) while **6** has higher value (3.18) probably because of forming the intermolecular hydrogen bond between hydroxy group and benzothiazole nitrogen. This can possibly lead to unspecific bonds in brain such as to white matter and therefore it could lead to decrease the resolution. However, logPrange of ,**7,** and ,**8,** is optimal for BBB crossing. All ,**6**,**7**, and ,**8**, have shown almost the same good affinity for Aβ in post mortem human AD brain homogenates, human AD brain and transgenic AD mice. ,**6**,**7**,**8** displayed good results in comparison with[^{125}I]-IMPY ,**9,** in binding studies. Ki (Ki= IC50 / (1 + L/Kd)- IC50 = inhibition constant 50%, L = concentration of radioactive ligand , Kd = dissociation constant of the radioactive ligand) of 6,7and 8 was 18,8 ± 3,8 ; 11,5 ± 3 and 11,2 ± 5 (PiB – 2,5 ± 5). Most promising seemed to be the 5-hydroxy-2-(4´-[^{11}C]methylamino)-1,3-phenylbenzothiazole ,**7**, because of the best brain uptake of all three isomers (2 min *per injection)* and the brain clearance which was 8 times faster than PiB, although all three compounds seemed to be suitable candidates for *in vivo* AD diagnosis.[11]



6

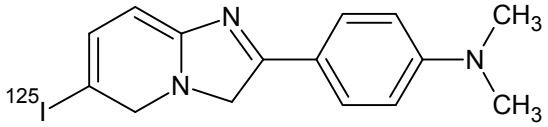


7



8

Figure 4.Pitsburg compound-B isomers 4-hydroxy-2-(40-[^{11}C]methylamino)-1,3-phenylbenzothiazole (6), 5-hydroxy-2-(40-[^{11}C]methylamino)-1,3-phenylbenzothiazole (7) and 7-hydroxy-2-(40-[^{11}C]methylamino)-1,3-phenylbenzothiazole (8)



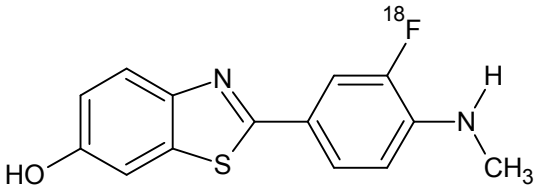
9

Figure 5. [^{125}I] IMPY

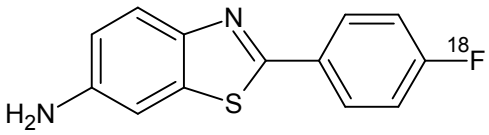
Although PiB and its 11C analogues showed great accessibility as PET imaging agents in AD diagnosis one of its major drawback is the short radioactive half-life of [^{11}C] (20.4 min). Therefore a rapid synthesis and immediate administration to patient is needed. On this account there is a need for several other radionuclides with longer half-live. [^{18}F] seems to be an optimal option for radiotracing due to its radioactive half-live (109.8 min). It allows imaging Aβ outside of hospitals with cyclotron and allows enough time for radiotracing.

One of option to get [^{18}F] labelled PiB analogues,investigated with several research groups, was adding the F atom in the structure in position thought not to negatively affect its Aβ plaques binding ability. First published [^{18}F] fluorinated compound was 3′-[^{18}F]-*N*-methyl-2-(4′-methylaminophenyl)-6-hydroxybenzothiazole called [^{18}F]GE067 or flutemetamol,**10**[12]. The binding equivalency of PiB and **10** has been proven in human *postmortem* brain. Although **10** gave higher resolution due to higher half-live of [^{18}F][13].

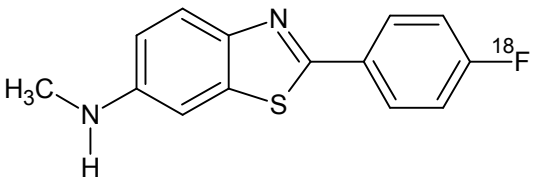
Furthermore Serdons*et al*. have developed three new potential PET agents similar to PiB structure ,**11**,**12**,**13**,. They contain the fluorine atom directly attached to the 2-phenyl ring and bear methyl substituted or unsubstituted amino group inthe benzothiazole part. Introduction of amino group in molecule was thought to increase binding affinity to Aβ plaques.It has been found that these congeners have a low Ki value (≤ 10 nM) in [^{125}I]-IMPY binding competition experiment with human AD brain homogenates. This means they have high affinity to Aβ plaques. However, the most promising molecule for human administration among them was found the (6-amino-2-(4′-[^{18}F]fluorophenyl)-1,3-benzothiazole),**12**,. Compound **12,** showed standardised uptake values (SUVs) in the range from 4 to 6 in animal PET studies in normal rats [14].



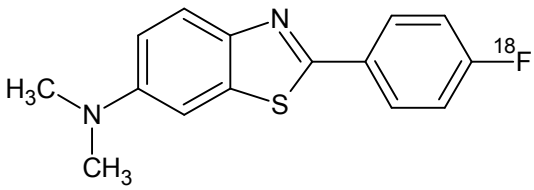
10



11



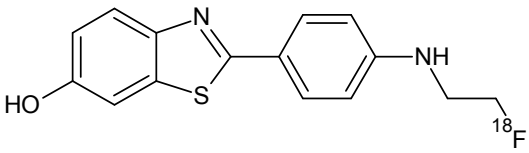
12



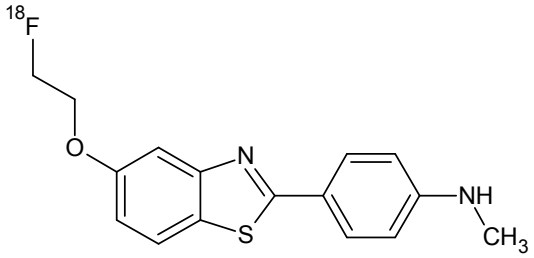
13

Figure 6.Flutemetamol (10) , 6-amino-2-(4′-fluorophenyl)-1,3-benzothiazole (11) , 2-(4′-fluorophenyl)6-methylamino-1,3-benzothiazole (12) and 2-(4′-fluorophenyl)6-dimethylamino--1,3-benzothiazole (13)

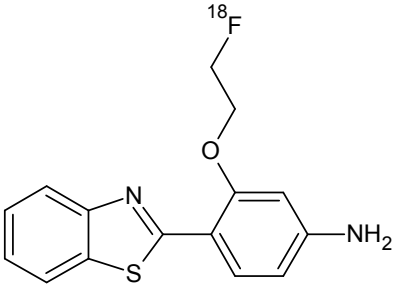
Some others potential [^{18}F] labelled phenylbenzothiazoles suitable for PET were synthesised by the introducing of the [^{18}F]-2-fluoroethyl group on PiB and its analogues. The closest one is [^{18}F] 2-[4′-(2-fluoroethyl)aminophenyl]-6-hydroxybenzothiazole (FBTA, **14**) Binding affinity of FBTA for Aβ was shown to be higher than that of PiB in human post mortem brain in a displaceable manner to the same binding sites [15]. Neumaier *et al.* have developed three [^{18}F] labelled benzothiazoles and observed them as potential PET agents: , **15**, [^{18}F] 2-((2′-(2-fluoroethoxy)-4′-amino)phenyl)benzothiazole ,**16,**
and [^{18}F] 2-(3′-((2-fluoroethoxy)-4'-amino)phenyl)benzothiazole) ,**17**.A high initial brain uptake and rapid washout and optimal *Log P* was observed for all of them. Although similar pharmacokinetics were comparable, the binding assay (using synthetic Aβ fibrils) shoved significant difference between, 16 and **17**, and ,**15**,. **16** and **17** displayed K_i range of ≥ 600nM. The K_i of ,**15** , however was within optimal range (7,2 nM). The binding affinity of **15** was also confirmed by autoradiographic assay using slices of AD patient brain.Therefore**15** appeared to be useful as *in vivo* PET agent [16].



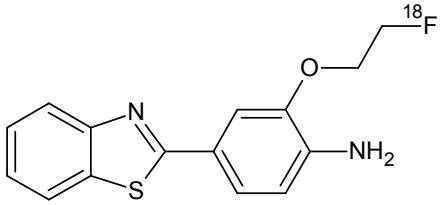
14



15



16

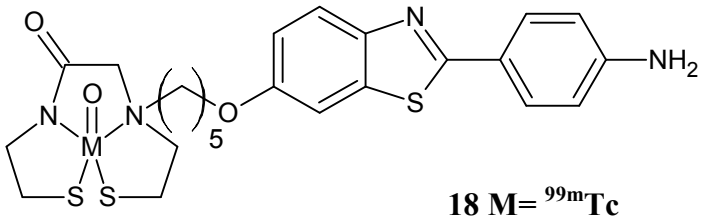


17

Figure 7. [^{18}F] 2-[4′-(2-fluoroethyl)aminophenyl]-6-hydroxybenzothiazole (FBTA, 14), ([^{18}F] 2-(4′-(methylamino)phenyl)-6-(2-fluoroethoxy)benzothiazole (15), [^{18}F] 2-((2′-(2-fluoroethoxy)-4′-amino)phenyl)benzothiazole (16) , [^{18}F] 2-(3′-((2-fluoroethoxy)-4′-amino)phenyl)benzothiazole (17)

As shown previously the most research development was made in the field of PET technique and SPECT is lagging behind. The most widely radionuclide used for SPECT is 99mTc. This isotope has several advantageous properties ($t_{1/2}$= 6 h, Ec= 140 keV) which makes it useful for SPECT. In 2007, Xiangji Chen *et al.* synthesised benzothiazole aniline (BTA), analogue conjugated with monoamine-monoamide (MAMA) labelled with 99mTc ,**18**,. 99mTc-MAMA-BTA .The optimal log P range to cross BBB is within 1.5–2.7 with 2.1 as optimal. High lipophilicity is also thought to be a crucial property to binding the amyloid plaques. However high log P value causes higher blood proteins binding ability which could possibly lead to high blood background. Therefore MAMA, as a less lipophilic ligand, was chosen to overcome this problem. The 5-carbon chain was thought not to affect the BTA binding properties by the chelating moiety. The rhenium analogue Re-MAMA-BTA, **19**, was synthesised (rhenium complexes have generally the same biodistributional properties) and its Aβ binding properties were tested in transgenic mouse and AD patient. The 99mTc BTA compound was investigated to cross BBB. It has shown high initial uptake and medium washout [17].

Furthermore several new 99mTc labelled 2-arylbenzothiazoles have been synthesised and *in vitro* evaluated in recent years. Jinhe Pan´s group have developed 24 new compounds bearing benzothiazole moiety labelled with 99mTc. Even almost of them have shown log P in optimal range (1.59–3.53) and they displayed Aβ fibrils binding affinity in range within Ki= 30–617 nM[18]. In future it would be better to have compounds with better Ki value (≤ 10).

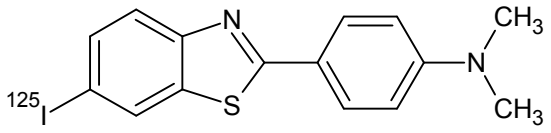


18 M= ^{99m}Tc

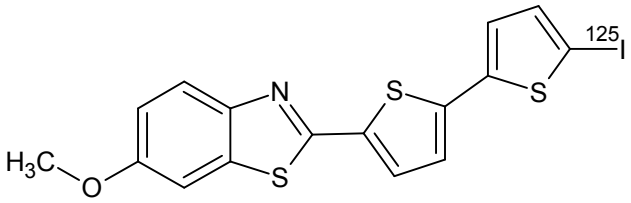
19 M= Re

Figure 8. 99mTc-MAMA-BTA (18) and Re-MAMA-BTA (19)

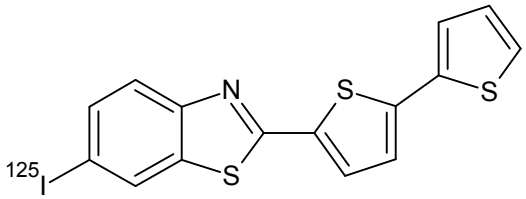
Another radionuclide used for SPECT is ^{123}I and ^{125}I. The 2-[4′-(dimethylamino)phenyl]-6-iodobenzothiazole (TZDM) labelled with ^{125}I ,**20,** was synthesised and *in vitro* evaluated as possible imaging agent. It was tested in *post mortem* Down´s syndrome (DS) human brain. This was chosen since patients with DS after fifth decennium develop Aβ plaques (because of the triplication of responsible gene locus). TZDM was shown great Aβ visualisation ability, high initial brain uptake and rapid washout. However this ligand labelled also the white matter which can negatively affect the visualisation [19, 20]. Cui, M. Chave developed several new compounds based on 2-arylbenzothiazole structure. They used bithiophenyl instead of phenyl to get higher BBB crossing ability. Two of those compounds [^{125}I]-2-(5´iodo-2,20-bithiophen-5-yl)-6-methoxybenzo[*d*]thiazole,**21**, and [^{125}I]-2-(2,20-bithiophen-5-yl)-6-iodobenzo[*d*]thiazole [^{125}I],**22**, have been successfully labelled with ^{125}I and evaluated for Aβ visualisation using [^{125}I] TZDM as a reference compound. In *in vitro* autoradiography study with AD mice brain slices these compounds showed excellent binding ability with low background for both compounds. They also showed excellent plasmatic stability but unfortunately the decomposition of, **21**, in liver homogenate was about 71% in 2 hours. Although the Aβ affinity was similar for both compounds, the pharmacokinetics was completely different. **21** showed poor results whilst displayed high initial uptake and rapid washout in normal mice. Therefore seems to be a hot candidate for future research [21].



20



21



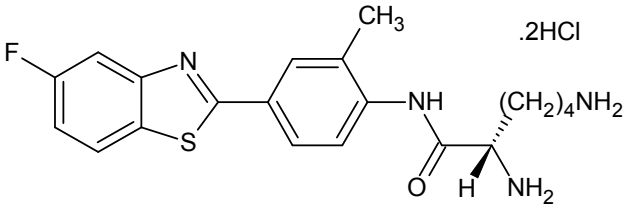
22

Figure 9. 2-[4′-(dimethylamino)phenyl]-6-iodobenzothiazole (TZDM) , [125I]-2-(50-iodo-2,20-bithiophen-5-yl)-6-methoxybenzo[*d*]thiazole and [125I]-2-(2,20-bithiophen-5-yl)-6-iodobenzo[*d*]thiazole

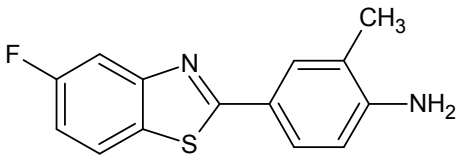
1.4. Benzothiazoles in cancer

Cancer, disease characterised with uncontrolled cell growth, is currently among the major cause of death worldwide. Estimated over 12.7 million new cases of cancer were in year 2008 worldwide. In UK, 324.579 cases of cancer were diagnosed in year 2010. In Czech Republic 78 846 new cases of cancer were diagnosed in 2009 and 27.680 people died from cancer in that year. Breast, lung, prostate and colon cancer represent 54% of all cancer cases [22,23].

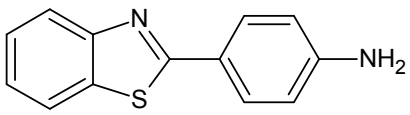
There is a number of benzothiazole compounds displaying anticancer activity reported in recent literature. In particularly interesting are the 2-phenylbenzothiazoles which have displayed anticancer activity particularly (not limited) against ovarian, breast, lung, renal, and colon cancer. The most promising one at the present time is Phortress ,**23**, which is currently being evaluated in clinical trials. **23,** is a prodrug of 2-(4′-amino-3′-methylphenyl)-5-fluorobenzothiazole (5F 203, **24**) which has recently exhibited unusual antiproliferative activity against NCI 60 cells *in vitro*. The development of **23** begun from Shi, D.F. and BradshawT.D. *et al.* They synthesised a series of 2-(4′-aminophenyl) benzothiazoles. Among these 2-(4′-aminophenyl)benzothiazole(CJM 126, **25,**) showed unusual biphasic and dose response growth inhibition against MCF-7 breast carcinoma cells [24].



23



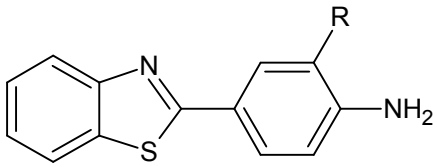
24



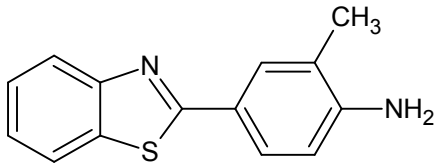
25

Figure.10. Phortress(23), 5F 203 (24) and CJM 126 (25)

Because of favourable features of **25** the structure-activity studies were carried out. It was found that benzothiazole nucleus is absolutely crucial for the anticancer activity and any structural modification has dischemotherapeutic effect. Alkoxy and hydroxy substitution on benzothiazole nucleus without additional substitution on 4′-aminophenyl ring was shown to decrease the activity. Particularly interesting was substitution on position 3′ on 4-aminophenyl ring. Oxidation of amine into nitro group in 3′substituted derivativesof ,**25,** was proved to decrease the activity. However introduction of methyl, ethyl,bromo ,iodo and chloro substituents on position 3′ (**26**) showed enhanced activity against MCF-7 and MDA 468 cell lines displaying GI50 values below 0,0001 μM .3′ substitution with previously mentioned groups extended antitumor *in vitro* ability to breast, ovarian, renal, colon, melanoma and non small cell lung . In contrast, introduction of cyano and hydroxyl substituent decreased the activity. In a similar way , the 3′,5′ dichloro and dibromo substitution as well as 3′- chloro, 5′- methyl were found to be less active than **24**. In particular, the 3′-methyl substituted **25** (DF 203, **27**,) has shown outstanding activity in both *in vitro* and *in vivo* studies [24,25,26,27].



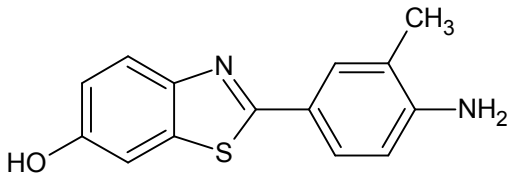
26



27

Figure. 11.2-(4′-aminophenyl)benzothiazole with favourable substitution R = Me,Cl,Br,I(26)and DF 203 (27)

As mentioned previously, DF 203 possessed interesting anticancer capability. A correlation between DF 203 uptake from nutrient media and anticancer efficiency was found[28] . It has been discovered that the lysates of cells whose growth was inhibited with DF 203 exhibit specific induction of cytochrome P4501A1 (CYP1A1),which is responsible for detoxication of noxious substances. (Therefore a conclusion was made that CYP1A1 plays crucial role in **27** mechanism of action [29]. CYP1A1 induction is connected with arylhydrocarbon receptors (AhR) activation. **27** creates an aduct with AhR , translocates to the nucleus ,dimerises with arylhydrocarbon receptor nuclear transporter and subsequently induces transcription of responsible genes [30].It was therefore assumed that several electrophile compound created by CYP1A1 induction can damage sensitive nucleophilic centre in cell DNA. Unfortunately, CYP1A1 also catalyzes detoxication of, **27,**. Main detoxication product is the 6OH 203, **28**, which was found in nutrient media of cells incubated with, **27,**. This metabolite was shown to decrease anticancer ability of DF 203. Fluorination hasrecently been proven method to avoid unwanted hydroxylation. A series of mono and difluorinated analogue of ,**27,** was made , in which particularly 5F 203, **24**, exhibits superior antiproliferative activity against several breast cancer cell lines *in vitro* and MCF-7 cell line *in vivo* [31,32]. However the main drawback of, **24**,was the low water solubility. To overcome this problem several water-soluble prodrugs of, **24,** in which the amino group was acylated with alanineor lysine were made. Among of them the superior antitumor properties were found for lysyl-amide of 5F 203 (Phortress), **23**,[33]. Scheme of Phortress mechanism of action is shown in figure 13.



28

Figure. 12. 6-hydroxy DF 203 (28) – the main methabolite of DF 203

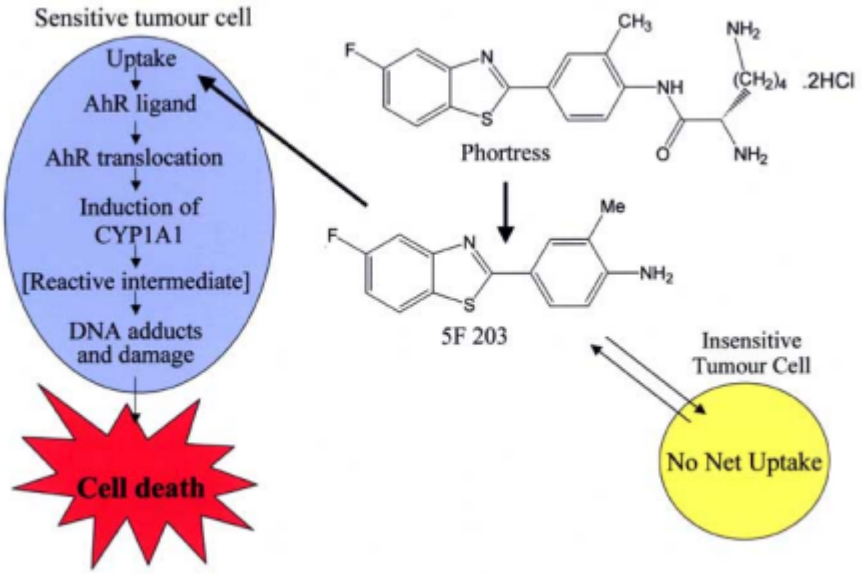
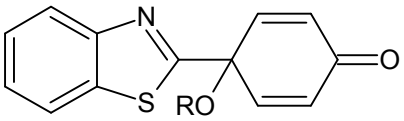


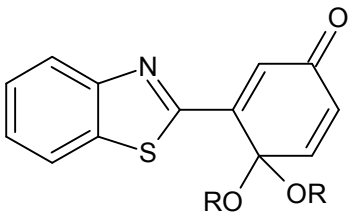
Figure 13.Phortress mechanism of action Scheme (taken from T.D. Bradshaw and A.D. Westwell)[33]

A series of 2-phenylbenzothiazoles had been synthesised before **27** and related compounds were established. A series of benzothiazole substituted quinol derivatives were synthesised and their anticancer ability was looked into. The precursor phenols were oxidised to its related hydroxycyclohexa-2, 5-dienones (**29**, **30**) using [di(acetoxy)iodo]-benzene (DAIB) as specific oxidative agent. The compounds were evaluated *in vitro* in the HCT-116 , HT29, MCF-7 and MDA468 human cell lines for possible antiproliferative ability. Among them, 4-benzothiazol-2-yl-4-acetoxycyclohexa-2,5-dienone (**29**, R = Ac) showed superior ability against both colon (HCT-116 , HT29) cell lines. All tested compounds exhibit higher anticancer ability against HCT-116 in comparison to HT29. In most tested cell lines the oxidized compoundswere found to be more active that starting phenols, however in MDA468 (oestrogen receptor-negative) the 2-(4-hydroxyphenyl)benzothiazole and 2-(3-hydroxyphenyl)benzothiazoleexhibitedbiphasic dose-dependent activity[34].



R= Ac, Me, Et, Pr, $CH_2C{=}CH_2$

29



R= Me, Et

30

Figure 14.Derivativesof 4-benzothiazol-2-yl-4-hydroxycyclohexa-2,5-dien-1-one (29) and of 3-benzothiazol-2-yl-4,4-dihydroxycyclohexa-2,5-dien-1-one (30)

Since the fluoroderivatives of 2-(4´aminophenyl)benzothiazole were shown to possess higher activity than their non-fluorinated analogues, a series of new fluorinated congenersbearing cyano , or a (trimethylsilyl)ethynyl), **31**, **32**, **33**, substitution in3´ position have been synthesised by Hutchinson and co-workers. Those compounds were *in vitro* evaluated in MCF-7 and MDA 468 cell lines. The highest potency in this evaluation was shown with5-fluoro substituted compounds (**31c**), **32c**) and **33c**)) in all Three groups. However the **32c**) was remarkably less active than the others once. The superior anticancer ability against MCF-7 and MDA 468 showed with the compound **31c**) (GI_{50} (μM): MCF-7 = 0.0057; MDA 468 = 0.0078). This compound was subsequently evaluated in NCI (NCI 60-cell line) showing noteworthy activity in for example NCL-H322M (non-small lung cancer), HCC 2998 (colon cancer) and IGROV1 (ovarian cancer) [35].

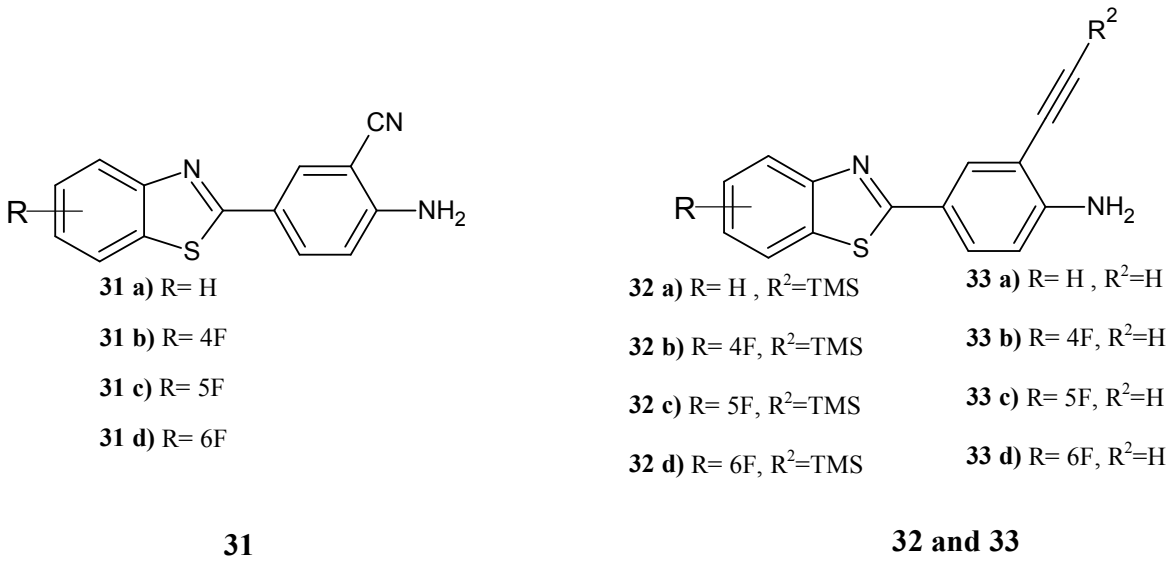
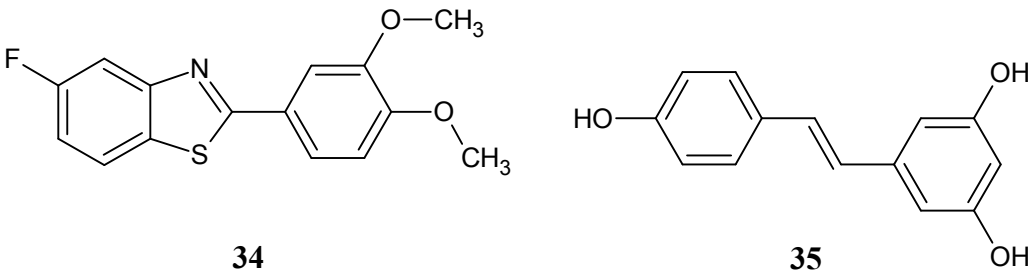


Figure 15. 2-(4´aminophenyl)benzothiazole bearing substitution in 3´ position and their fluorinated analogues

A series of 2-phenylbenzothiazole bearing hydroxyl in the phenyl ring was synthesised by Mortimer *et al.* Their activity was tested *in vitro* in breast cancer cell lines MCF-7 and MDA 468 and colon cancer cell lines KM12 and HCC 2998. Among them,5-fluoro-2-(3,4-dimethoxyphenyl)benzothiazole(GW 610, 34,) showed superior activity against tested cell lines. Activity against both tested breast cancer cell lines was comparable with recently tested compounds with high anticancer activity,**24**, and,**27**, (GI_{50} < 0,0001 μM). Surprisingly, better activity was shown in colon cancer lines, where,**34**, displayed superior activity against HCC 2998 cell line (GI_{50} < 0,00025 μM) compared to that of,**24**, and,**27**, (GI_{50} > 100 μM). It showed also superior activity among all tested compounds in KM12 cell line (GI_{50} = 0.29 μM). Minor structural changesin ,**34**, led to loss or at least decreased activity of this compound. Unfluorinated analogue of ,**34**, almost completely lost its efficiency against tested cell lines. Similarly the moving of the methoxy group into5´-position decreased activity to micromolar range , on the other hand addition of another methoxy group in 5´position (3´,4´,5´-trimethoxy) retained the GI_{50} range for breast cancer lines. The second most promising compound in this series was the 2-(3,4-diethoxyphenyl)-5-fluorobenzothiazole which showed GI_{50} for MCF-7 line in range of 0,0007μM whereas seemed not to be applicable in colon cancer lines. Additional testing of,34,in NCI showed remarkable potency in non-small lung cancer (NCL- H450 and NCL- H226),colon cancer (HCC 2998) and leukemia (SR line). It is

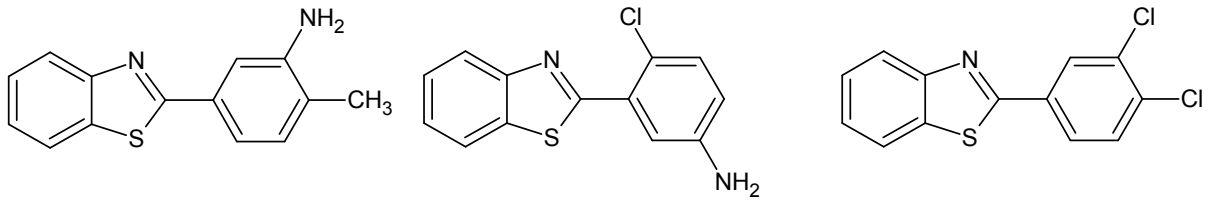
apparent that CYP1A1 plays a role in anticancer mechanisms of,**34**, However,resveratrol,**35**, inhibitor of CYP1A1 was found not to affect the anticancer ability of ,**34**. Moreover in sensitive colon cancer lines no expression of CYP1A1 proteinwas found.It is therefore apparent that the whole effect cannot be explain by induction of CYP1A1 [36].



34 **35**

Figure 16. GW 610 (34) and resveratrol (35)

As can be seen from preceding section, anticancer mechanism of action of 2-phenylbenzothiazoles involves not only CYP1A1 activation. Another possible mechanism represents inhibition of topoisomerase, enzyme responsible for topological arrangementof DNA and is supposed to be one of crucial factor in cell division. Many of currently used anticancerdrugs, target topoisomerases.Camptothecin and related compoundsinhibitTOPO I, whilst doxorubicin and etoposideare inhibitors of.TOPO II [37]. For that reason a series of various substituted 2-phenylbenzothiazoles bearing substituent on the phenyl ring was synthesised. The compounds were evaluated for their *in vitro* as possible inhibitors of TOPO II and the anticancer efficiency for certain cancer cell lines was studied as well. For this use the A549 (lung cancer), Col2 (colon cancer), SNU-638 (stomach cancer), T1080 (fibrosarcoma cancer), and HL-60 (myeloid leukemia) were chosen. Remarkable antiproliferative effect was shown with compounds 36 and 37 for HL-60) cell line. The cytotoxicity of compound 36 (GI_{50} = 0,02 μM)was even higher than that of doxorubicin(GI_{50} = 0,041 μM) In TOPO II inhibition assay compounds 36and 38 showed equal ability in comparison with etoposide whilst 37exhibit only average activity [38].



36 **37** **38**

Figure 17. 2-(3´amino-4´methylphenyl)benzothiazole (36) , 2-(5´amino-2´chlorophenyl)benzothiazole (37) and 2-(3´-4´dichlorophenyl)benzothiazole (38)

Chapter 2

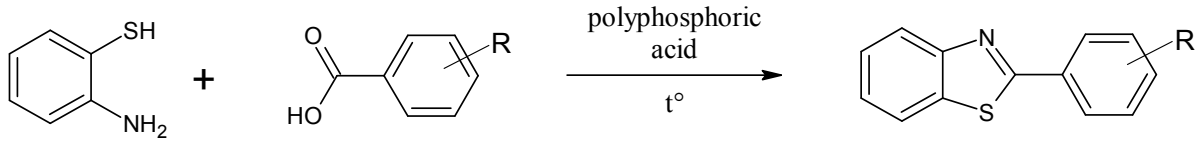
2.1. Solution phase synthesis of 2-phenylbenzothiazole

Currently, the most reported synthetic ways leading to 2-phenylbenzothiazoles relate to those thatare non-substituted on benzothiazole ring. However, as stated previously, the most effective ones for medical use are substituted on both benzothiazole and phenyl ring. Therefore development of benzothiazole substituted 2-phenylbenzothiazole method is more challenging at the present time. In this chapter several methods related topreparation of 2-(subst. phenyl)benzothiazoles will be overviewed.

2.1.1 Procedures using 2-aminothiophenoland related disulfides

2.1.1.1 Via carboxylic acid and derivatives

Most of published synthetic methods are based at 2-aminothiophenol condensation with different type of compounds using several agents as a cyclisation mediators. Early example of arylbenzothiazole synthesis involves condensation of aminothiophenol,**39**, and appropriate benzoic acid or related derivate,**40**, in the presence of high boiling solvent (polyphosphoric acid) to give substituted 2phenylbenzothiazole,**41**, [39,31]. This method is still useful at present days.

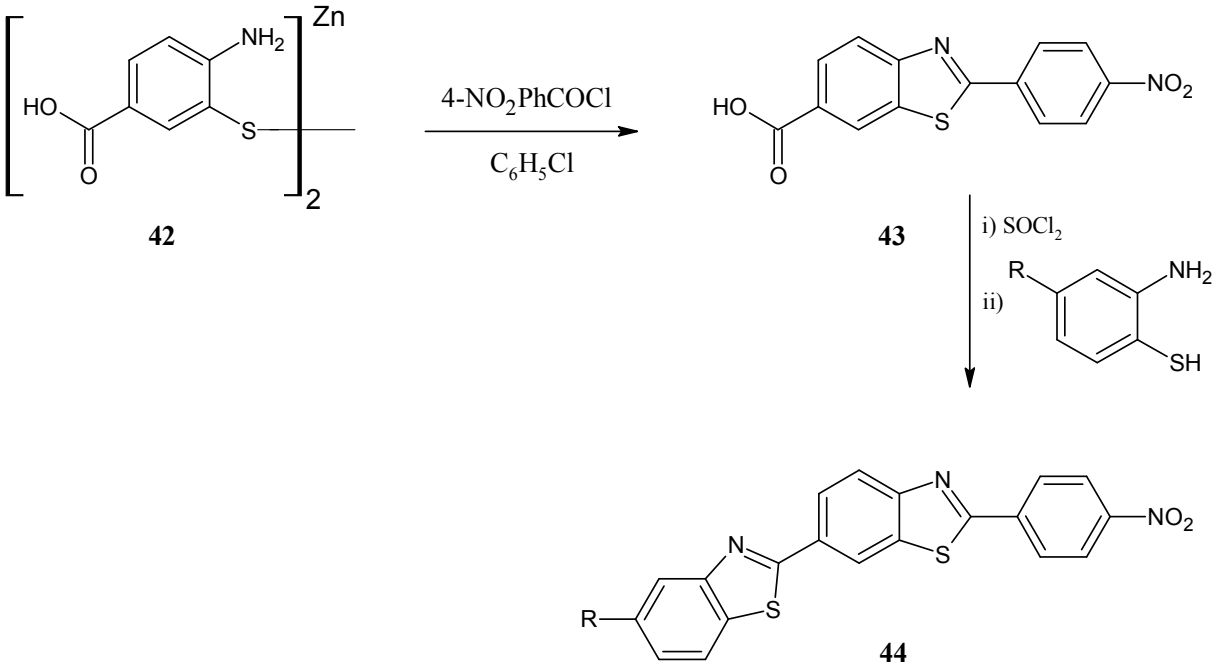


39 **40** **41**

Scheme 1. Synthesis of 2-arylbenzothiazoles from 2-aminothiophenol and substituted benzoic acids and their derivatives

Benzoic acid could be converted to benzoylchlorides via thionyl chloride reaction. The related chlorides can react with **39** to produce appropriate 2-phenylbenzothiazoles using basic solvents (pyridine, triethylamine, NMP ...)[40,41]. Mild condition seems to be the major advantage of this method [42].

The synthetic method using benzoic acids and theirderivatives leading to 2-phenylbenzothiazoles substituted on benzothiazole ring have recently been reported. The zinc salt of 2-aminothiophenol disulfide-5-carboxylic acid, **42**, with 4-nitrobenzoylchloride in chlorbenzeneyielded2-arylbenzothiazole-6-carboxylic acid, **43**, as a product. This product was subsequently derivatised using $SOCl_2$ to give related acylchloride which was in the next step converted to final dibenzothiazole, **44**, [43].

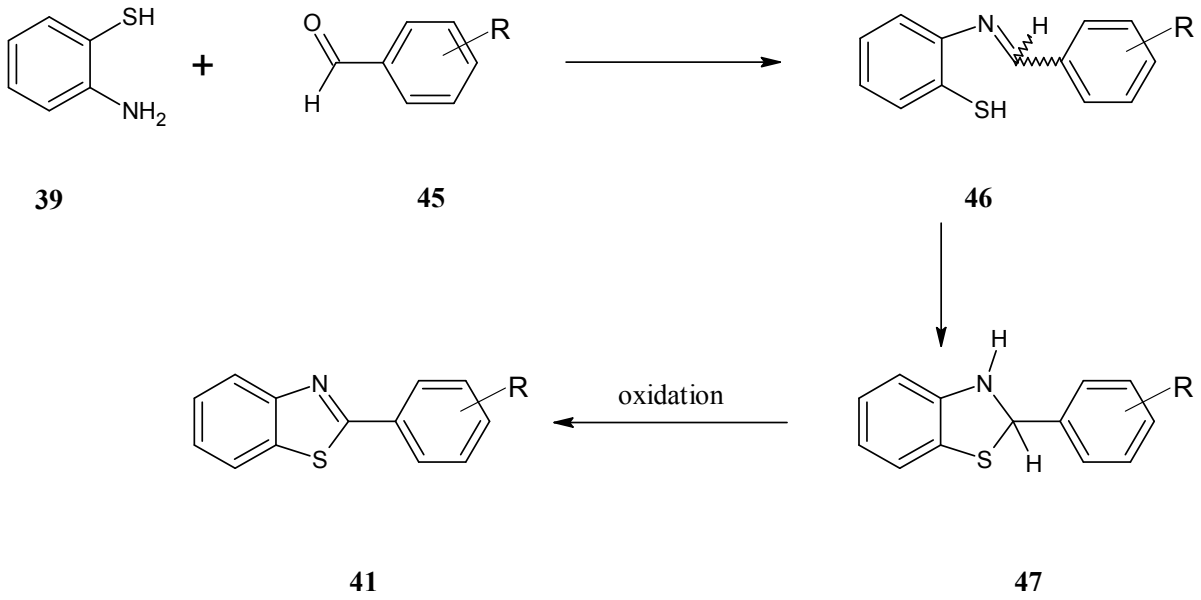


42 **43** **44**

Scheme 2. Synthesis of bibenzothiazolesfrom a disulfide precursor

2.1.1.2. Via benzaldehydes

Appropriate substituted benzaldehydes,**45**, react with 2-aminothiophenol,**39**, in either basic or acidic conditions to produce related 2-phenylbenzothiazole,**40**,. The initial step of the reaction is thought to be the Schiff's base,**46**, formation. This base subsequently cyclisises to dihydro intermediate,**47**,. Further oxidation (spontaneous or using oxidative agent) leads to related 2-phenylbenzothiazole. Several authors have recently described this reaction in additional oxidising agent free conditions using DMSO [44,45] ,ethanol [36] and nitrobenzene [46] as solvent.

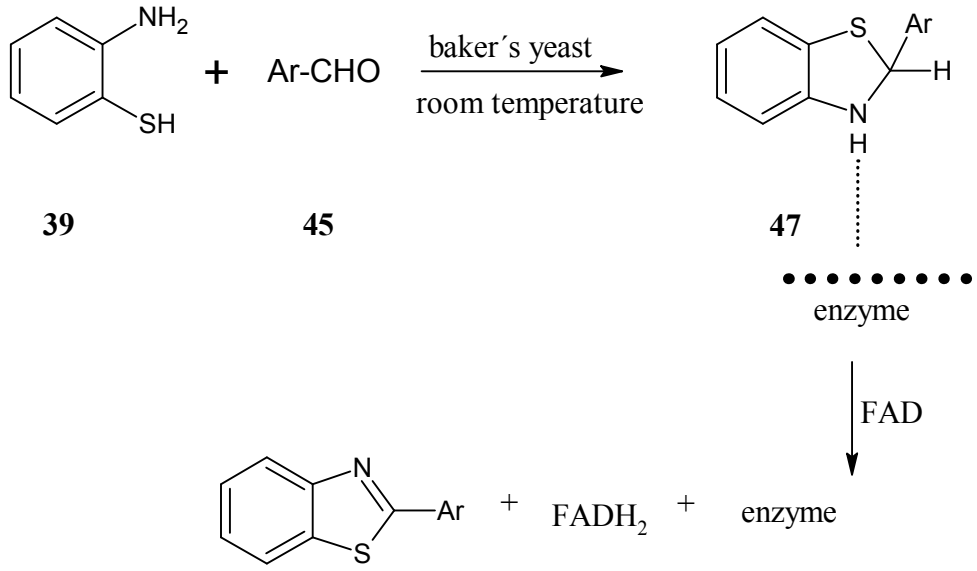


Scheme 3. Reaction mechanism of reaction of 2-aminothiophenol and benzaldehydes

A large number of methods describing arylbenzothiazole ring formation using various reactants and catalysts have been reported. For example, the method using *p*toluenesulfonicacid (*p*TSA) [47], acetic acid [48] or promoted by basic solvent such as pyridine [49] have been described. Umesh and co workers developed a convenient and environmentally friendly method leading to 2-phenylbenzothiazoles using baker's yeast (S*accharomyces cerevisiae*) as catalyst. Baker's yeast has previously been used for number of various organic reactions due to their favourable properties. However, they cannot be used in aqueous condition for dehydrogenation reaction. Therefore several reactions using various organic solvents were carried out to find optimal conditions. Furthermore, biocatalysis in organic solvent exhibits a number of advantages, such as ability to carry out reaction which is impossible in aqueous solvent, easy product recovery from the solvent, insolubility of enzyme in the solvent which allows their easy recovery and reuse. The optimal solvent was found to be CH_2Cl_2 (DCM) which exhibit optimal yields of desired products. The described method gave from 57% to 84% yields. The baker's yeast is well known source of enzymes. Therefore it was assumed that these enzymes can possibly accelerate the cyclocondensation either by forming an initial enzyme–2-aminothiophenol non-covalent complex or an enzyme–aldehyde complex, leading to dihydro intermediate, **47,**.

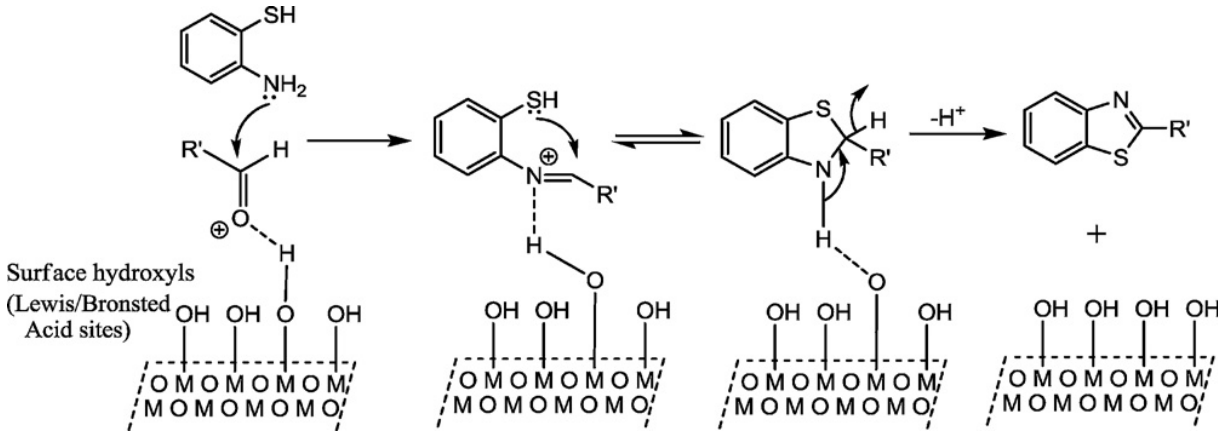
The coenzymes, nicotinamide adenosine dinucleotide or flavin adenosine dinucleotide-dependent oxidoreductases available in baker's yeast can possibly catalise the aromatisation,Scheme 4.,.

The major advantages of described method seemed to be incomparably milder condition (room temperature) in comparison with previously mentioned reaction, using non-expensive catalysis and chemical stability of all substituents under described condition [50].



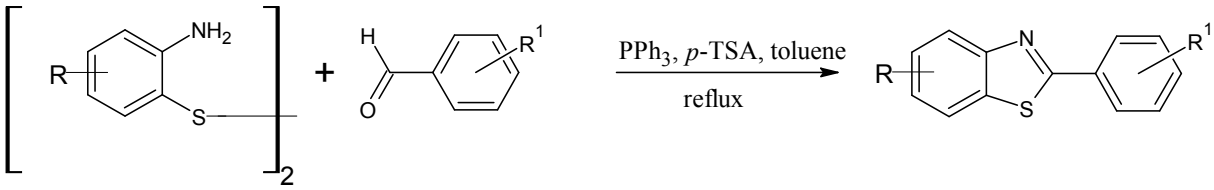
Scheme 4. Possible reaction mechanism of baker's yeast mediated cyclocondensation of 2-aryl-benzothiazole

Another method to 2arylbenzothiazole employing 2-aminothiophenol synthesis was reported by Bandyopadhyay *et al.* They developed new, rapid and environmentally friendly method achieving very good yields. They used mixed nanocrystalic Al2O3–Fe2O3,Al2O3–V2O5 and Al2O3–CuO as a heterogenous catalysts for cyclocondensation of appropriate benzaldehydes and aminothiophenol. The proposed mechanism is presented in Scheme 5. The miscelaneous metal oxides contain Lewis acid sites and Bronsted acid sites in addition to basic surface sites. The aldehyde carbonyl oxygen reacts with these acidic sites by forming intermolecular hydrogen bond and therefore supports the Schiff base formation. Optimal catalyst reaction range was found to be 5% of the entry mass. Major advantages of reported method are high yields, environmental benignity, relatively cheap starting material for catalyst formulation and the catalyst reusability (After the reaction was complete the catalyst was washed off with EtOH/H_2O and dried at 100°C. This catalyst was found not to lose any of its catalytic ability) [51].



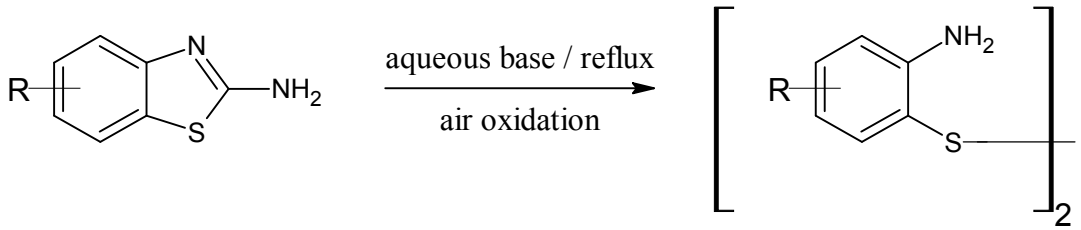
Scheme 5. 2-arylbenzothiazole forming on mesoporous mixed metal oxide catalyst (taken from P. Bandyopadhyay *et al.*[51])

One of the most useful method, reported in this thesis, is a formation of 2-phenylbenzothiazoles substituted on both benzothiazole and benzenering,**49**, from bis(2-amino-4-X-phenyl)disulfides ,**48**,and various substituted benzaldehydes,**45**, has recently been developed by Mortimer et al[36] .Authors used triphenylphosphine(PPh_3) and *p*-TSA as a catalyst **Scheme 6.**. Bis(2-amino-4-halophenyl)disulfides were obtained by hydrolytic cleavage of corresponding 6-substituted derivatives of 2-aminobenzothiazoles,**50**, under basic condition in aqueous solvent following recently published method (**Scheme 7.**[52,53,54]. 6-substituted 2-aminobenzothiazoles are readily available by Sigma-Aldrich in sufficient purity. Previously mentioned reactants were treated under reflux condition using toluene as solvent. After the reaction was complete the products were purified by column chromatography to remove PPh_3 oxide by-product obtaining from moderate to good yields. The main advantage of this method is using relatively stable and non-toxic disulfide instead of aminothiophenol(seriously affects aquatic environment).



48 **45** **49**

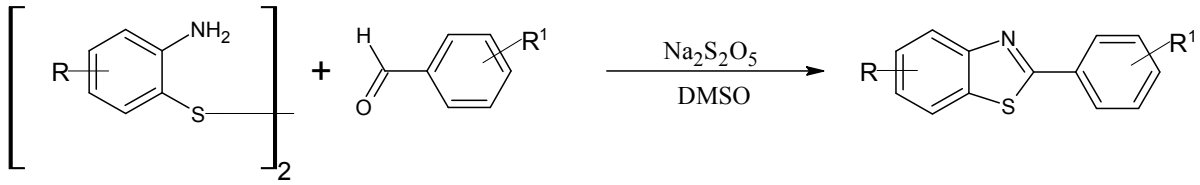
Scheme 6.Synthesis of benzothiazoles49 using PPh_3, *p*-TSA as a catalyst and toluene as a solvent.



50 **48**

Scheme 7. Hydrolytic cleavage of 2-aminobenzothiazole leading to correspondingbis(2-aminophenyl)disulfide

Although an efficient and in comparison to 2-aminothiophenol methods environmentally benign, the major downfall of previously settled method is the need a column chromatography for product purification. To overcome this obstacle a new method using different substituted disulfides as the reactant was developed by Weekes et al [55]. The starting point of this reaction was synthesis of various substituted 2-aminothiophenol disulfides, **48**, bearing different (both electron donating and electron withdrawing) substituents (**R**) following foregoing method **Scheme 7.** The subsequent step was the reaction of resulting 2-aminothiophenol disulfide and appropriately (both electron donating and electron withdrawing) substituted ($\mathbf{R^1}$) benzaldehyde, **45**, using the non-toxic and inexpensive oxidant sodium metabisulfite ($Na_2S_2O_5$) in DMSO at 120 °C.



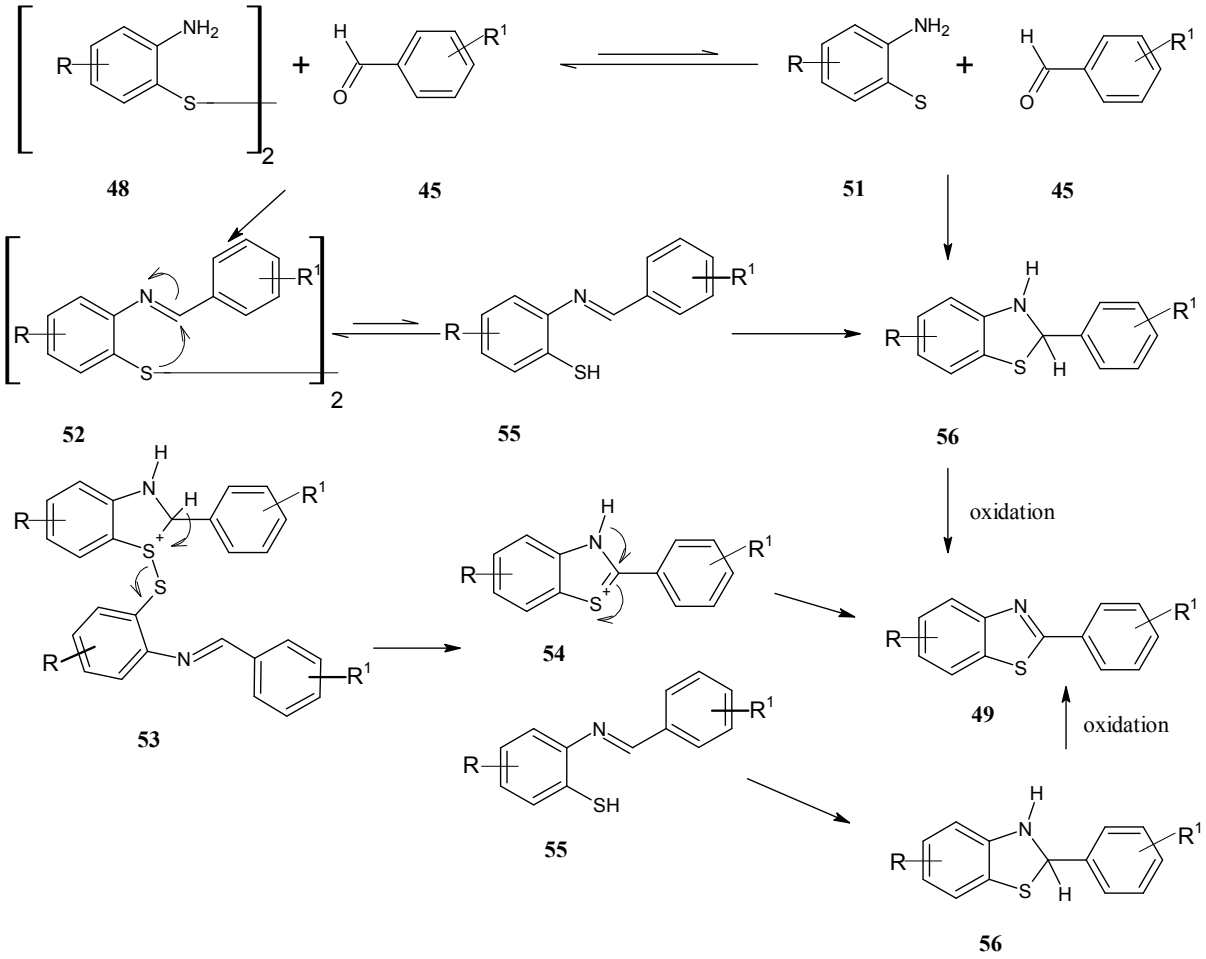
48 **45** **49**

R= 5-F; 4-F; 5-Cl; 5-EtO; 5-MeO; 5-NO2; H

R1= 4-OMe; 3-OMe; 3,4-di-OMe; 3,4,5,-tri-OMe; 3-OH; 4-OH; 3-F;-3-Cl; 4-Br; 4-CN; 3-NO2; 4-NO2

Scheme 8. Synthesis of substituted 2-phenylbenzothiazoles using $Na_2S_2O_5$ as oxidant.

The reaction time was usually from 40 to 90 minutes. After the reaction was completed, the straightforward purification including water addition, filtration of the precipitate and washing out the traces of by-product and solvent with water was carried out. Recrystalisation of product to obtain pure compound was necessary in several cases[55].

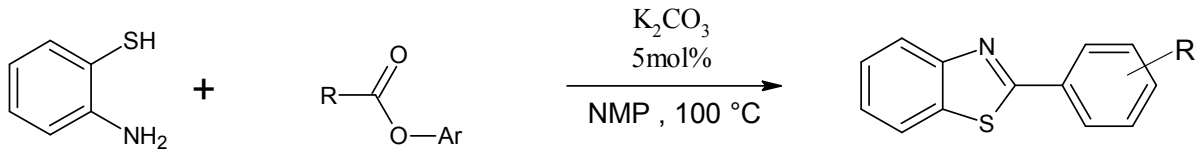


Scheme 9. Possible mechanism for formation of substituted 2-phenylbenzothiazoles under 'oxidising' conditions

The assumed mechanism of this reaction,**Scheme 9.**, includes reaction of amino group of aminothiophenoldisulfide, **48**, with benzaldehyde, **45**, forming initially disulfide adduct **52**. Further cyclization of **52**can possibly lead to **53** which subsequently cleaves to give tautomer of the 2-phenylbenzothiazole product, **54**, and iminothiophenol, **55**. Intermediate **55** can either form the dihydro intermediate **56** or be re-oxidised to **52** which can form the product via **53** and **54**. Another possible way to form the desired product includes the disulfide bond splitting and forming equilibrium of disulfide,**48**, and aminothiophenol, **51**, in DMSO (although unsubstituteddisulfide was found to be stabile in DMSO). It is therefore assumed that even small amount of water in DMSO could lead to SO_3 forming. SO_3 can facilitatethe thiophenol formation. **56** which subsequently cyclisises to **49**. The major superiority of this method is definitively the simple and short time purification without column. The other advantage includes low price of sodium metabisulfite and environmental benignity of method[55].

2.1.1.3 Via esters

The synthesis of **41** using appropriate esters, **57**, and K_2CO_3 as a cyclocondensation catalyst has been reported. Authors used *N*-methyl-2-pyrrolidone (NMP),member of group of polar aprotic solvents, as solvent and the reaction have been performed at 100°C. This method is suitable for acid and base sensitive groups because of relatively mild condition of this reaction **Scheme 10**[56].



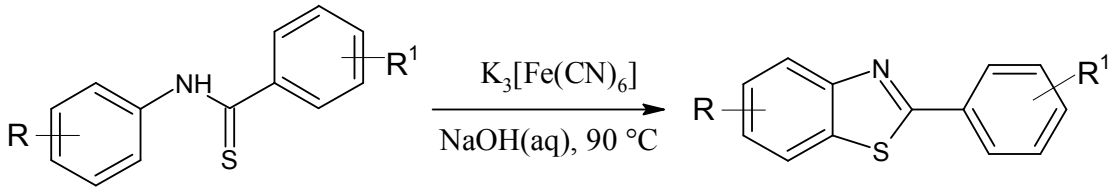
39 **57** **41**

Scheme 10. Synthesis of substituted 2-arylbenzothiazoles using phenolic esters

Similar reaction using orthoesters to form 2-alkylbenzothiazoles under solvent-free conditions has been reported. In this reaction the catalytic amount of $ZrOCl_2.8H_2O$ has been used to form the desired product [57].

2.1.2. Via thioamide cyclisation

Another highly important method to 2-phenylbenzothiazoles is a thioamide, **58**, cyclisation. This method represents valuable way to get 2-phenylbenzothiazoles substituted on both benzothiazole and phenyl ring, **49**, and therefore is frequently published by groups which are interested in 2-phenylbenzothiazole synthesis [58,59,54]. Several cyclisation agents can be used for thioamide cyclisation, however, the most used one is the Jacobson cyclisation mediated by potassium ferricyanide, $K_3[Fe(CN)_6]$ in aqueous solution of sodium hydroxide Scheme 11..



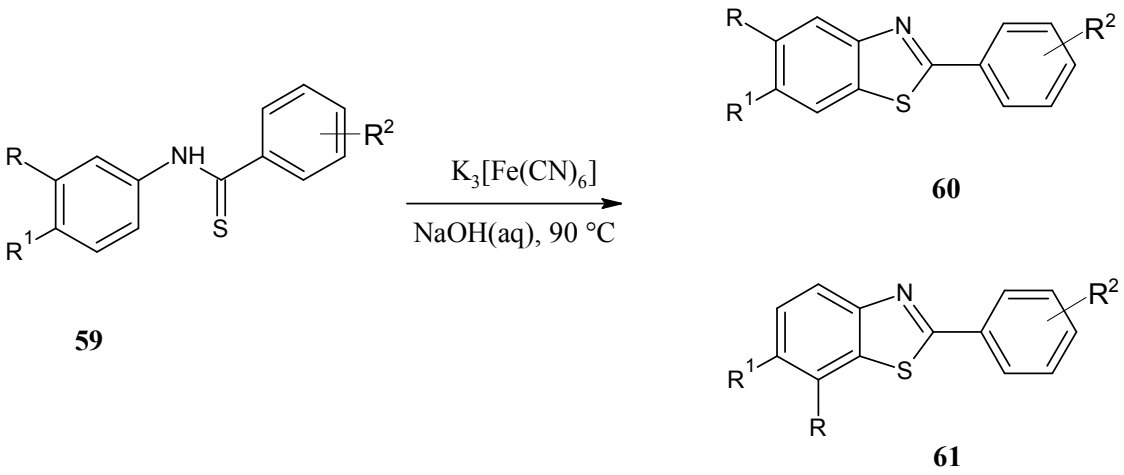
58 **49**

Scheme 11. Jacobson cyclisation mediated by $K_3[Fe(CN)_6]$/NaOH(aq)

This method usually gives about 60% yield. Several authors [58,59] use small amount of EtOH for wetting the thioamide go get better results.

A number of proceduresincluding thioamide cyclisation using different agent has been reported in recent years. One of reported method consists in thecyclisation mediated by elemental bromine[94]. It has been found to require reflux for approximately 2.5 h in chloroform. The main downfall of this method is the strong oxidative activity, toxicity of vapour and difficult manipulation with Br_2. To overcome foregoing the new method employing elemental iodine was developed by Downer-Riley and Jackson [60]. Iodine is available in solid form and is less harmful than bromine. Apart from that I_2 was previously found to mediate several organic cyclisation such as thiols. Sodium hydride/iodine mediated cyclisation was reported in previous article as well. In several cases it gave better results that iodine. This method is suitable for thioamides without an orthoalkoxy or ester group. Another method employed Dess-Martin periodinane (hypervalent iodine compound) as a suitable cyclisation agent [61]. It gave excellent yield (90%) in short time period)15min.)[62].

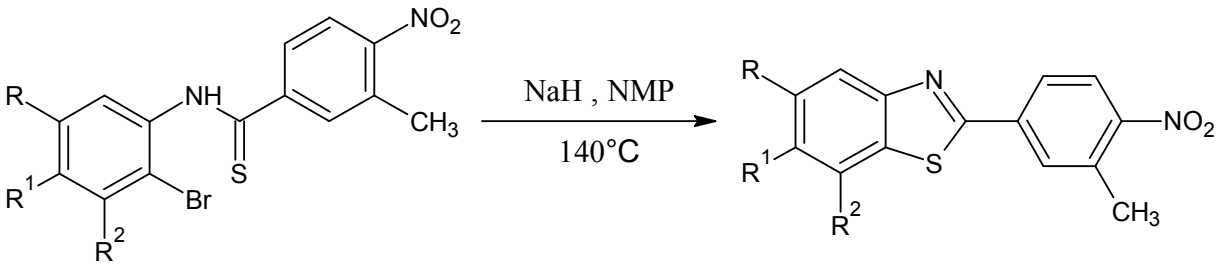
All previously reported methods have the same limitation. They are suitable for thioamides bearing substitution on *ortho* and *para* position considering the thioamide group. *Meta* and disubstituted **59** usually leads to regioisomers, **60** and **61**, Scheme 12.



59

Scheme 12. Isomers formation in disubstitutedthioamides

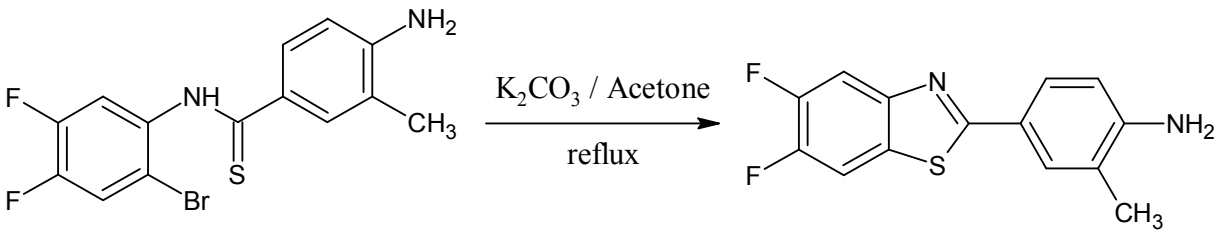
To overcome this drawback some new methods have been developed. Access to pure regioisomer was achieved in this case by introduction of a bromine atom in *ortho*position. The next step involves cyclisation of the thiobenzanilides using sodium hydride in NMP at 140°C, Scheme 13.The yields obtained by this method were from 73 to 91 %. Another and even more favourable method includes milder conditions than for previously mentioned. In this case K_2CO_3 in refluxing acetone has been used to form the desired product via an alternative route,Scheme 14,. As can be seen in Scheme 14, the milder conditions are suitable for amino group [63].



62 63

	R	R1	R2
a	F	H	H
b	H	H	F
c	F	F	H
d	Br	H	H

Scheme 13.Regiospecificthiobenzanilidecyclisation using NaH



64 **65**

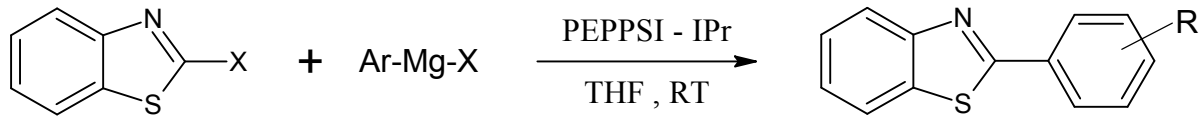
Scheme 14. Regiospecificthiobenzanilidecyclisation using K_2CO_3 in refluxing acetone

2.1.3. Via carbon-carbon bond forming

A number of transition metal-catalyzed carbon-carbon bond forming reactions between carbon in position 2 on benzothiazole ring and leaving group-containing aryl rings (Ar-X) have been reported in recent years.

2.1.3.1. Via Palladium-Catalyzed Coupling of Grignard Reagents (Kumada-Tamao-Coriu Reaction)

An easily employed and versatile Kumada-Tamao-Coriucrosscoupling reaction using 2-halobenzothiazole, **66**, and a range of arylmagnesium bromides catalyzed by PEPPSI (pyridine-enhancedprecatalyst, preparation, stabilisation and initiation) has been reported [64].



66 **40**

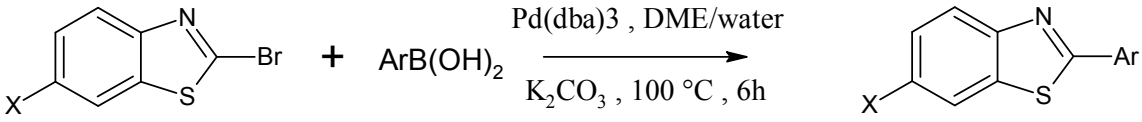
X= Cl,Br

Scheme 15.BiarylKumada-Tamao-Coriu cross-coupling reaction catalyzed by PEPPSI-IPr ([1,3-Bis(2,6-Diisopropylphenyl)imidazol-2-ylidene](3-chloropyridyl)palladium(II) dichloride)

Related coupling using 2-chlorobenzothiazole with phenyl magnesium chloride under manganese chloride catalyzed conditions has been reported as well. This reaction takes an advantage from milder condition to form **40**[65].

2.1.3.2. via Palladium-Catalyzed Suzuki-Miyaura Coupling

The synthesis of 2-arylbenzothiazoles employing arylboronate coupled with 2-bromobenzothiazoles, **67**, has been reported by Majo and co workers [66]. This method provided in general moderate (35% – 74%) yields. The corresponding arylboronic acids have been used as well for this reaction Scheme 16. This palladium catalyzed coupling has been further extended to cross-coupling with benzothiazole-2-thioether, mediated by copper (I) thiophene-2-carboxylate [67]. In a further extension of traditional Suzuki-Miyaura coupling , the palladium catalyzed and microwave mediated coupling of number of arylboronic acids with 2,6-dichlorobenzothiazole in aqueous dioxane has been described [68].



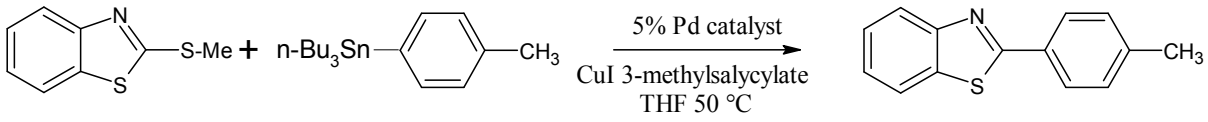
67 **68** **69**

X= H,OMe

Scheme 16.Biaryl coupling of 2-bromobenzothiazole with various boronic acids

2.1.3.3. via Stille-Type Coupling

Coupling of 2-tributylstannylbenzothiazole as a coupling partner for aryl bromides/iodides under palladium catalyzed conditions has been reported in number of papers in recent years [69,70]. Masahiro and Lanny described various palladium catalyst catalyzed copper (I)-mediated cross-coupling of (benzothiazolyl)(methyl)thioethernrbo 2-methylsulfanylbenzothiazol, **70**, with aryl stannanes, **71**, using mild condition to give 2-arylbenzothiazoles in a good yield. Reported method provides a useful complement to the recently described and related coupling of heteroaromatic thioethers with boronic acids [71].



70 **71** **72**

Scheme 17. Palladium catalyzed copper (I) -mediated coupling of benzothiazole-2-thioether with aryl stannane

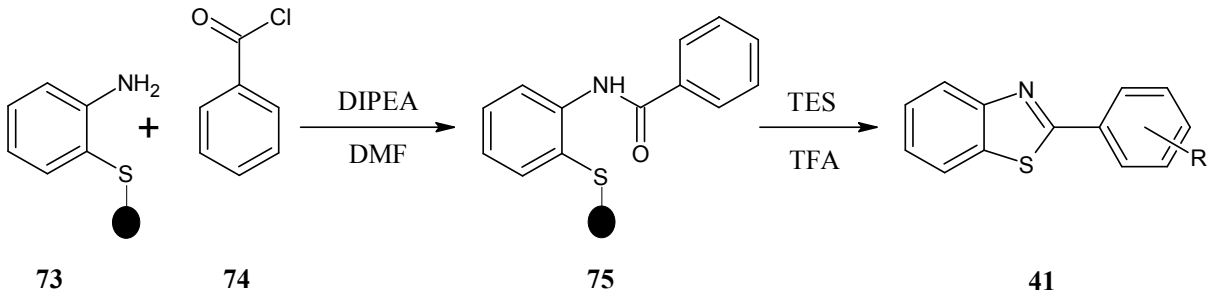
2.2. Solid-phase synthetic method leading to 2-phenylbenzothiazoles

Solid phase synthesis represents a valuable method for parallel synthesis of libraries of benzothiazole for future *in vitro* screening. Solid phase and in particular polymer-supported synthesis represents a convenient method taking advantage especially from easy by-product removing. The polymer can be easily filtered of , which makes this method rapidly in comparison with majority of solution phase method (there is usually a requirement for column chromatography to isolate the desire product in sufficient purity as stated in previous chapter). A number of methods employing solid phase to synthesise small libraries of 2-arylbenzothiazoles have been demonstrated in recent years. Here I report the most useful of them.

2.2.1 Synthesis starting from polymer-bound starting compound

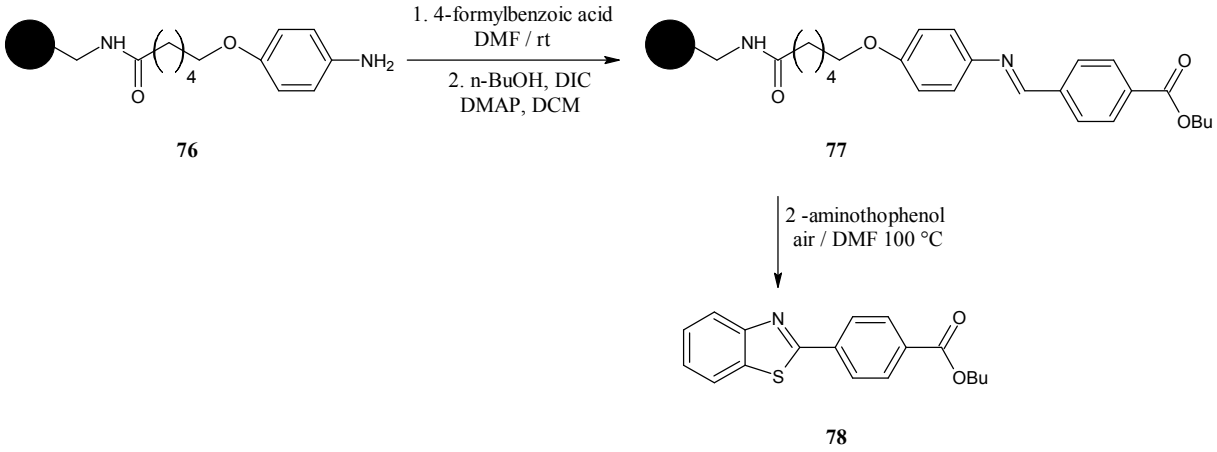
As written previously the solid phase method showed several logistic advantages in comparison with traditional solvent based method. For example the solid phase parallel synthesis of 2-arylbenzothiazoles using trityl resins has been reported. Mourtas et all.reported useful synthesis of 2-phenylbenzothiazoles from 2-aminothiophenol using various trityl resins. In the first step the 2-aminothiophenol was bound to the polymer

through its thiolgroup. Resulting polymer bound 2-aminothiophenol, **73**, was acylated with both aliphatic and aromatic carboxylic acids chlorides, **74**, achieving 2-N-acylaminobenzenethiols, **75**,. Resulting compounds were cleaved from the resins by reaction with trifluoracetic acid in DCM. The last step of reaction was cyclisation in MeOH or dithiotrethiol in DMF Scheme 18[72]. Another paper reporting method employing trityl resin was demonstrated by Choi *et al*. [38]. Several methods using Wang resin instead of trityl resin were reported as well [73,74].



Scheme 18. Solid phase synthetic method using trityl resin achieving 2-phenylbenzothiazole

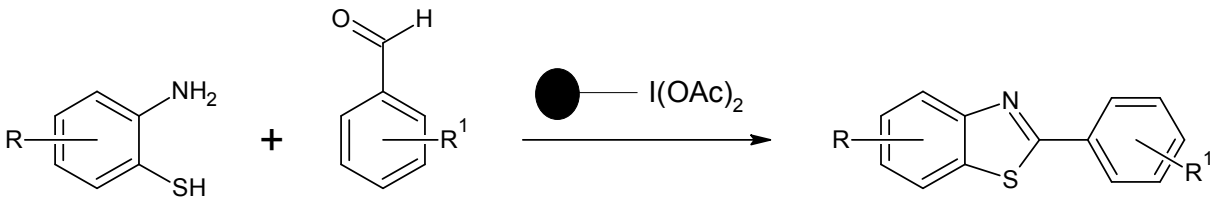
Hioki *et al.* have developed new convenient methodology to benzothiazole using traceless 4-alkoxy-aniline linker, **76**,. The desired product was released from the polymer using imine exchange followed by air-oxidation Scheme 19. In most cases the yield was up to 60%. Authors also investigated recycling of the polymer after reaction. It has been found that the recyclation is limited due to formation unwanted by-product and therefore the yields decreased by 10-20% in every recycling step [75]. A similar reaction using Wang resin-bound esters with various 2-aminthiophenols in methanesulfonic acid upon microwave irradiation has been reported as well [76].



Scheme 19.Polymer-supported synthesis of 2-phenylbenzothiazoles using a traceless aniline linker

2.2.2 Synthesis starting from polymer-bound additive oxidant

In most of solid phase synthetic method either the aminothiophenol or the benzaldehyde (or related derivative) has been attached directly to the polymer. This seems to be the major drawback of this method because it limits the diversity of resulting library. Therefore a new strategy involving polymer-attached additive oxidant was developed. Atul Kumar and co workers reported new method to 2-phenylbenzothiazole using polymer-bound hypervalent iodine reagent poly[4-diacetoxyiodo]styrene (PDAIS), Scheme 20.. PDAIS is a selective oxidant that does not affect the aldehydic group. Resulting benzothiazoles were obtained in excellent yields up to 90%. Reported method takes advantage of easy removing of the polymer by filtration and also of the possibility of recovering the polymer support using hydrogen peroxide and acetic acid [77].



51 **45** **49**

Scheme 20. Polymer-supported formation of 2(phenyl)benzothiazoles using polymer bound hypervalent iodine(PDAIS) as selective oxidant .

In general polymer and polymer-supported synthesis exhibit a convenient methodology with number of advantages. These methods are fully suited for future synthesis of chemical libraries of benzothiazoles as potential candidates for number of medicinal use. Therefore ma and co-workers inspired by recent papers reporting solution phase synthesis of 2-phenylbenzothiazoles report a new improved methodology in ongoing part of this thesis.

Chapter 3

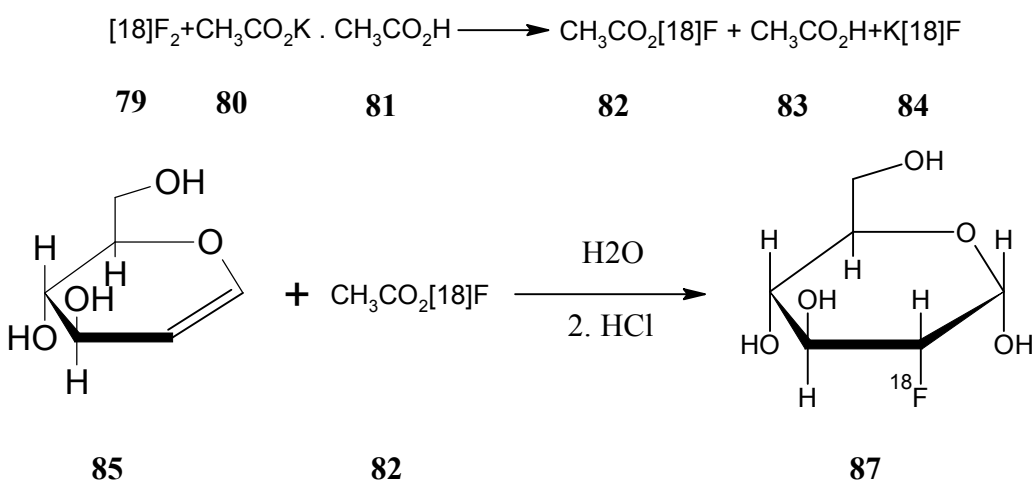
3.1. Radiolabeling with [^{18}F]

In this chapter I will give a overview about most used reaction and methods for labelling with [^{18}F] isotope. Following overview would not be an exhausting list of all currently used methods for radio-labelling. I will report the most useful methods important for purpose of this thesis.

[^{18}F]fluorine is a widely used radioisotope in PET. Due to high resolution and longer half-live than most of other PET isotopes it represents the ideal radionucleotide for PET. The half-live of 109.8 min. allows several logistic advantages as for example longer synthesis and purification and a lag between synthesis and administration to the patient. There are a number of synthetic methods leading to the incorporation of [^{18}F] to target molecule. The main fluorination strategies can be divided into two categories: a) electrophilic fluorination and b) nucleophilic fluorination.

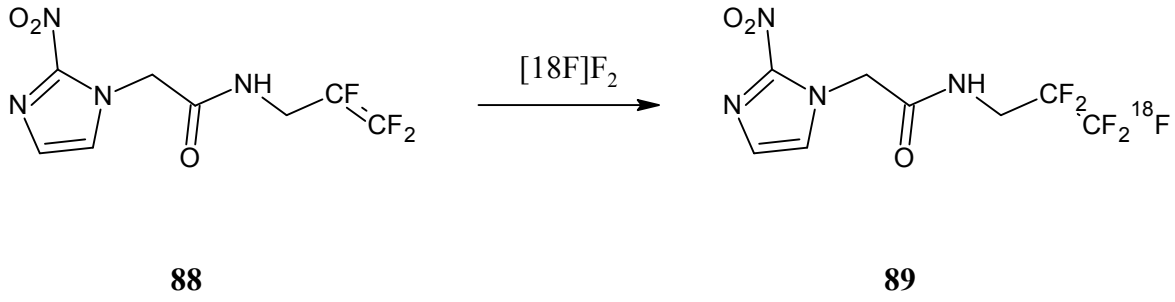
3.1.1. Electrophilic Fluorine-18 labeling reactions

A number of electrophilic [^{18}F] agents is currently used for radiolabeling. The most used one at the present time is [^{18}F]-F ($^{18}F_2$). Due to high reactivity of fluorine gas there is usually need for a control of its reaction. This is accomplished with either low temperature or by dilution using inert solvents. Another way to decrease the high reactivity of fluorine is conversion of fluorine into less reactive agent such as acetyl hypofluorite ($CH_3COO[^{18}F]F$). In recent article reporting synthesis of [^{18}F] labelled 2-fluoro-2-deoxy-D-glucose ([^{18}F]-FDG), **87**, the $CH_3COO[^{18}]$-F, **82**, was generated from potassium acetate /acetic acid complex. Once the complex was prepared it was placed into a stainless steel tube and the 18F fluorine was passed through this tube. The effluent was then passed to the reaction weasel containing D-glucal, **85**, dissolved in H_2O. Than the HCl was added subsequently followed by purification of [^{18}F]-FDG, Scheme 21.[78].



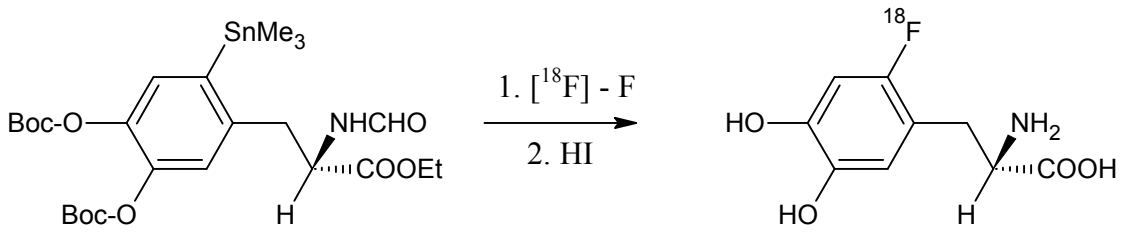
Scheme 21. Direct electrophilic fluorination using acetyl hypofluorite ($CH_3COO[^{18}F]F$)

Other derivatives that have been used as electrophilic fluorinating reagents are [^{18}F]fluoropyridones and [^{18}F]fluoro-*N*-sulfonamides [79]. Another example using [^{18}F]-F in trifluoracetic acid for direct electrophilic labelling of hypoxia marker EF5, **89**, has been reported [80].



Scheme 22. Fluorination of EF5 using fluorine

Both of foregoing reaction suffer from low specify of reaction and low specific activity. Better regiospecifity was achieved with using demethylation reaction. The most commonly used reagent at the present time aretrialkyltin or mercury group. Several papers describing synthesis of L-[^{18}F]fluoro-DOPA, **91**, from trialkylstannyl precursor, **90**, have been reported Scheme 23**.** [81].



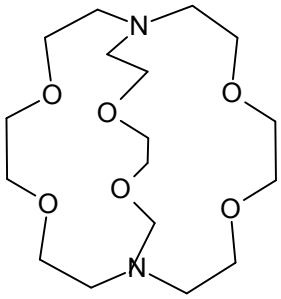
90 **91**

Scheme 23.L-^{18}F-fluoro-DOPA synthesis

3.1.2. Nucleophilic Fluorine-18 labeling reactions

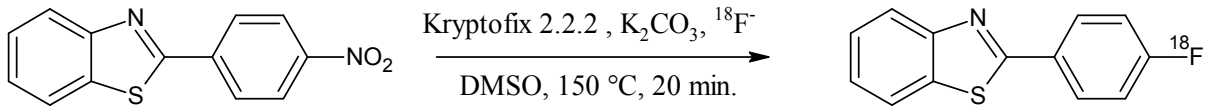
Nucleophilic[^{18}F]-fluorination is widely used to produce some of the most important PET radiotracers. The [^{18}F]$^-$is obtained as aqueous solution. However the aqueous fluoride is a poor nucleophile due the salvation. Therefore the [^{18}F]$^-$is trapped in an ion exchange column using potassium carbonate in a water/acetonitile solution as eluent. After elution the phase transfer catalyst Kryptofix 2.2.2 is added followed by water removal. The potassium cationt is trapped in Kryptofix making the fluoride ion exposed and highly nucleophilic in polar aprotic solvents [82]. An alternative to Kryptofix 2.2.2 is the tetrabutylammonium or tetraethylammonium bicarbonate (TBAB) [83].

Direct nucleophilic substitution on aromatic nucleus represents a simple one-step methodology to number of labelled aromatic compound. However there is need for several additional electro-withdrawing groups on the aromatic nucleus in *ortho* or *para* position. The presence of kryptand (Kryptofix 2.2.2 , **92**) and base (e.g., K_2CO_3 or $K_2C_2O_4$) is also required for this reaction. However there are several exceptions reported in recent papers. Serdons *et al.* have reported synthesis of 2-(4´-[^{18}F]fluorophenyl)-1,3-benzothiazole using 2-(4´-nitrophenyl)-1,3-benzothiazole as a precursor in presence of kryptofix 2.2.2 , K_2CO_3 and DMSO heating at 150°C for 20min Scheme 24.. The radiochemical yield was 38%[84]. Another paper reporting nucleophilic substitution of nitro group using fluoride ion to synthesise [^{18}F]-flumazenil showed that in several cases is possible to incorporate the [^{18}F]-fluoride ion into a weakly activated aryl position[85].



92

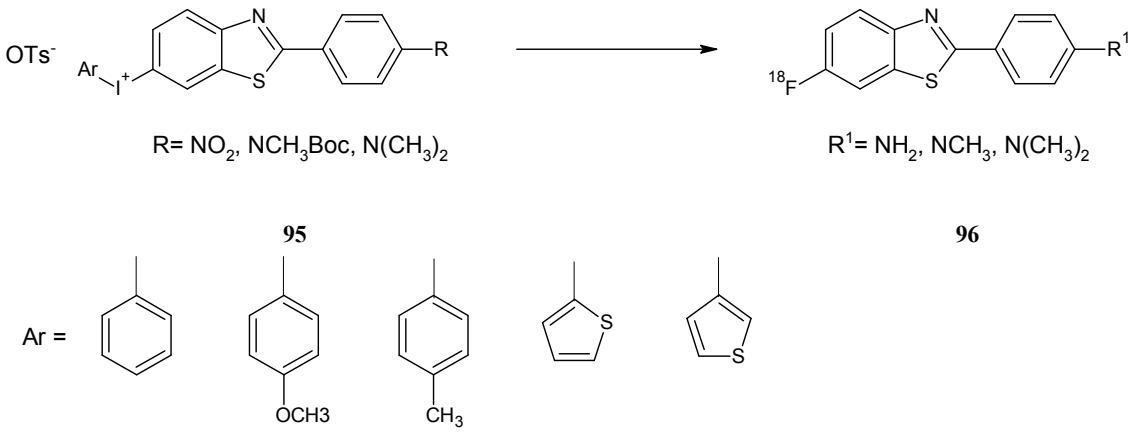
Figure 18.Kryptofix 2.2.2.



93 **94**

Scheme 24.Radiosynthesis of 2-(4-[^{18}F]fluorophenyl)-1,3-benzothiazole

The main downfall of aromatic nucleophilic substitution is their lack of applicability to electron rich aromatic rings. To overcome this problem the new method employing aryliodonium salt was introduced [86]. The main feature of aryliodonium salts used as precursors for labelling with [^{18}F] is the *ortho*-effect. Fluorine nucleophilic substitution is directed to ring bearing substitution in position *ortho*[82]. In the absence of *ortho*effect the fluorine incorporate into the less electon-rich aromatic nucleus. Due to this fact one of strategy for directing [^{18}F] labelling is to make one of the aromatic ring electron-rich. Hence they were several methods employing electron-rich rings such as p-methoxyphenyl, p-methylphenyl, and 2- or 3-thiophenyl on 6-position of benzothiazole ring Scheme 25.Thas was supposed to give superior efficiency to [^{18}F]fluorine labelling [87].



Scheme 25. Labelling with [^{18}F] using various aryiodonium salts.

Chapter 4

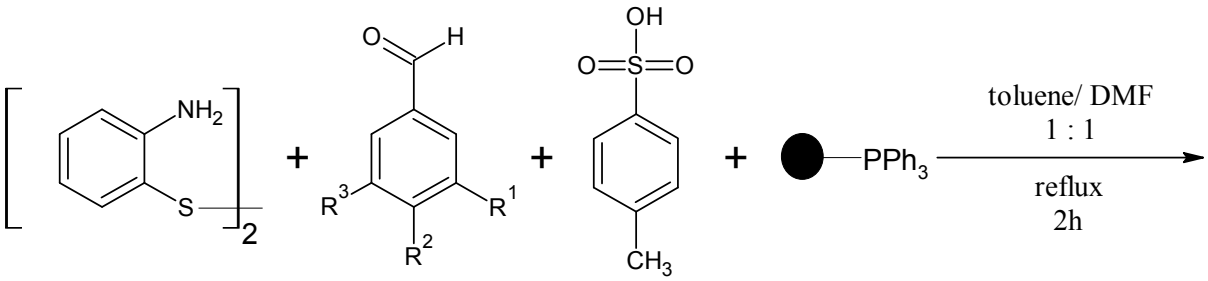
4. Results and discussion

4.1. Polymer-bound triphenylphosphine supported synthesis of 2-phenylbenzothiazoles substituted on phenyl ring

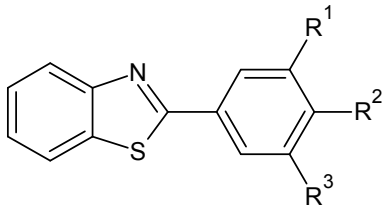
As stated in previous chapters, the most common way to 2-phenylbenzothiazoles includes either thermal or microwave promoted condensation of 2-aminothiophenol with benzaldehydes or benzoic acid derivatives. This reaction leads to the initial formation of dihydro intermediate converted in related benzothiazole final product by either *in situ* or more commonly by external oxidant. In the second chapter of this theses a synthesis employing bis(2-amino-4-halophenyl) disulfides was reported [55]. The mechanism has similar dihydrointermediate step as 2-aminothiophenol/benzaldehydes condensation. Apart of that a reaction using triphenylphoshine (Mortimmer *et al.*) has been reported in second chapter of this thesis as well Scheme 6[36]. This reaction gave particularly promising results however there was a requirement for column chromatography to remove PPh_3 oxide. Getting inspiration from polymer-bound hypervalent iodine additional oxidant reported in third part of this thesis, Me and my co-workers have developed new convenient method to 2-phenylbenzothiazoles using polymer-bound PPh_3.

4.1.2. General

Mixture of 2-aminothiophenol disulfide, substituted benzaldehyde, polymer-supported triphenylphosphine and *p*-toluenesulfonic acid in mixture of toluene and DMF was heated under reflux for 2h (Scheme 27). After 2 hours the mixture was cooled to r.t. and filtered through Celite® layer (see chapter 6. Experimental).[88] Results are shown in table 1.



97	**98**	**99**	**100**
1,0 mol equiv.	0,98 mol equiv.	0,2 mol equiv.	0,98 mol equiv.



101 - 106

Scheme 27. Standard procedure using polymer-bound triphenylphosphine

Table 1. Results of PPh_3 supported synthesis of 2-phenylbenzothiazoles

Compound No.	R^1	R^2	R^3	Yield (%)
101	O	OMe	H	60
102	OMe	OMe	OMe	71
103	NO_2	H	H	31
104	H	NO_2	H	13
105	H	Br	H	71
106	H	OH	H	58

Although in most cases yields up to 58% were achieved the nitro-substituted compounds gave low results. This is understandable considering electron-withdrawing effect of nitro group (especially in *o*-and *p*-position). To improve yields in the case of nitro-derivatives the purification work-up was slightly changed. Both 3- and 4-nitro derivatives had lower solubility in methanol then other reported benzothiazoles, but the solubility in acetone was good. Considering this fact acetone was used instead of methanol for washing the Celite®. As can be seen in table 2, better yields were obtained in both cases without changing the standard reaction condition.

Table 2. Using acetone for washing the product through Celite®

Compound No.	R^1	R^2	R^3	Yield (%)
103	NO_2	H	H	38
104	H	NO_2	H	61

To investigate the influence of polymer-bound PPh_3 to the reaction we compared two different cross-linked polymer-bound PPh_3. Reaction of 2-aminothiophenol disulfide and *p*-anisaldehyde using the same condition as stated in Scheme 27was chosen as a standard. In the first case I used 200-400 mesh, ~3.0 mmol/g loading and 2% cross-linked with divinylbenzene and in the second case 100-200 mesh, ~3.2 mmol/g loading cross-linked with divinylbenzene (I used the second one in the amount considering higher load of PPh_3 in comparison with ~3.0 mmol/g loading). However using different grade of polymer-bound PPh_3 did not significantly affect the reaction (± 5%).

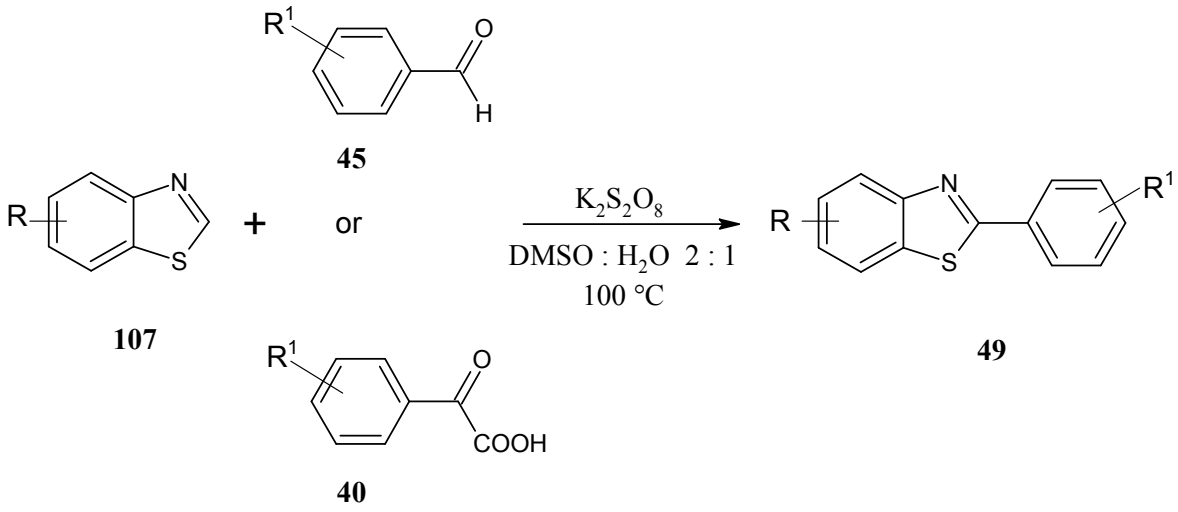
To further develop the reaction condition it was tried to use higher load of benzaldehyde. As standard was chosen reaction of 2-aminothiophenol disulfide and *p*-anisaldehyde using double load of *p*-anisaldehyde keeping the remaining condition as stated in Scheme 27. By using higher load of benzaldehyde the yield increased to 98% of pure product. Therefore higher amount of benzaldehyde seems to represent a favourable way to get higher yields.

4.1.3. Discussion

We developed a rapid and convenient synthesis of biologically relevant 2-phenylbenzothiazoles featuring substitution on phenyl. This method particularly takes advantage from avoidance of column chromatography and no requirement for protective group in case of sensitive groups such as OH (see table 1). Previously reported synthesis using 2-aminothiophenol disulfides bearing halogen substitution [88,53]and leading to the 2-phenylbenzothiazoles substituted on both benzothiazole and benzene ring indicated that this method is fully suited for parallel (libraries) synthesis of 2-phenylbenzothiazoles.

4.2. Sodium metabisulfite mediated synthesis of 2-phenylbenzothiazoles

Inspired by Zhiyong Yang *et al.,* which used potassium persulfate for 2-phenylbenzothiazole (up to 74% of yield) synthesis *Scheme* 28[89] it was tried to treat benzothiazole and 2-aminobenzothiazole with unsubstituted and substituted benzaldehydes in the presence of sodium metabisulfite as mild oxidative agent to get appropriate 2-phenylbenzothiazoles.

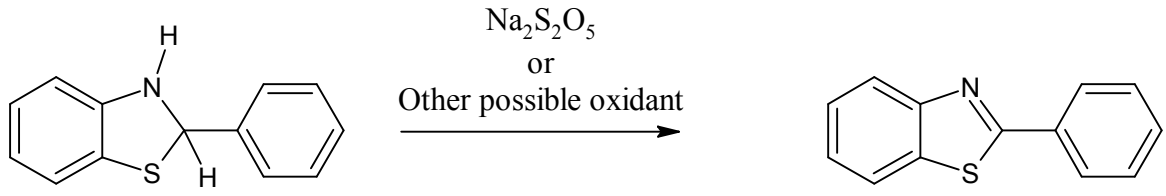


R - H, Cl, Me, OMe R^1 - F, Cl, Br, OMe, Me

Scheme 28. Potassium persulfate mediated oxidative condensation of benzothiazole with aryl aldehydes

Sodium metabisulfite was previously used for forming benzothiazole with benzaldehydes and 2-aminothiophenol disulphides (see chapter 2). Although sodium metabisulfite is weaker oxidant in comparison to $K_2S_2O_8$ it is still a perspective agent to mediate cleavage of thiazole side ring in benzothiazole compounds. Sodium metabisulfite has been previously proven by Weekes *et al.* as an oxidative agent for oxidation of 2,3-dihydro intermediate, crucial step of 2-phenylbenzothiazole

formulation Scheme 29[55]. However, we cannot use the same conditions as reported by Zhiyong *et al.* Because of water sensitivity of sodium metabisulfite we had to use anhydrous solvent. In spite of this fact even small amount in "anhydrous" solvent (especially DMSO is known for its high hygroscopic nature) could react with sodium metabisulfite affording sulphur oxide (SO_3) which can oxidise the intermediate dihydro product. This was supposed to improve yield of the reaction. Also 2-aminobenzothiazoles are easy to synthesise and in compare with benzothiazoles easily commercially available



47 **2**

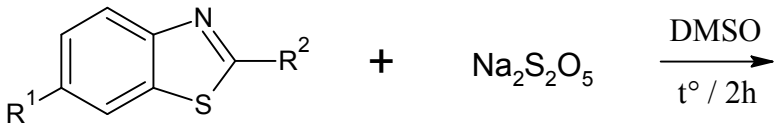
Scheme 29. Oxidation of 2,3-dihydro-2-phenylbenzothiazole

4.2.1. General

4.2.1.1. Procedure 1

We treated the substituted and unsubstitutedbenzothiazoles/aminobenzothiazoles with sodium metabisulfite in high-boiling solvent to find possible oxidative products (it was assumed that the sodium metabisulfite has ability to break the benzothiazole ring) of this compounds. Presence of some extra spot on TLC or extra shifts in NMR were thought to be the mark of oxidative product which can in reaction with several benzaldehydes lead to appropriate 2-phenylbenzothiazolesScheme 30.

Substituted/unsubstituted benzothiazoles, Scheme 30, were dissolved in dry DMSO. To the solution 0.5g sodium metabisulfite was added and the resulting mixture heated at 100 °C for 2 hours. The mixture was then cooled to the room temperature and poured into water following by extraction with diethylether. Organic layer was extracted with brine subsequently followed by drying over sodium sulfate and then evaporated. Unfortunately, all experiments at 100°C did not show any extra spot on TLC and NMR data were unsatisfactory as well. Therefore harsher condition (at least 120°C)were chosen for the following experiment (see table 3.)



108 **109**

Scheme 30. Benzothiazole/ 2-aminobenzothiazole reaction with sodium metabisulfite

Tab. 3. Benzothiazole/ 2-aminobenzothiazole reaction with $Na_2S_2O_5$ condition

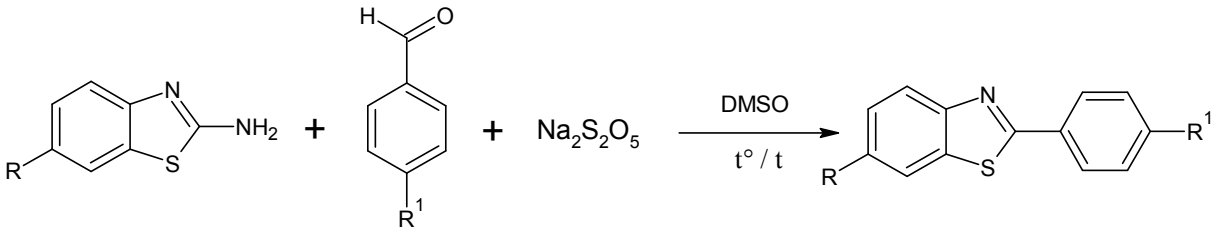
entry	R	R^2	time (h)	t(°C)	Result
1.	- H	- H	2	Reflux	no product
2.	- Br	- NH2	2	Reflux	product
3.	- H	- NH2	2	120	product

In the first case there were no other peaks in NMR spectra than the starting compounds peaks. Neither TLC showed any extra spots. In second and third case both TLC and NMR showed several new (we did not purify them) compounds in the reaction mixture. Therefore it was decided to use 2-aminobenzothiazole for following reactions.

4.2.1.2. Procedure 2

After successful experiments with 2-aminobenzothiazoles with $Na_2S_2O_5$ it was decided to continue with both 6-substituted and unsubstituted 2-aminobenzothiazoles (both commercially available). They were treated with both electron-donating and electron-withdrawing *para*-substituted benzaldehydes and sodium metabisulfite in dry DMSO at different temperature Scheme 31.

The 6-substituted/unsubstituted 2-aminobenzothiazole and appropriate para-substituted benzaldehyde were dissolved in dry DMSO. To the resulting mixture the sodium metabisulfite was addend and the mixture stirred at various temperatures (tab 4.) for 2 hours. After cooling to the room temperature the water (40ml) was added and the mixture was extracted 3-times with diethyl ether. Organic layer was washed 2-times with water following with washing with brine and subsequently dried over sodium sulphide and evaporated under reduced pressure obtained yellow unpleasant-smelling oil.



110 **45** **109** **111**

Scheme 31. 2-aminobenzothiazole reaction with p-substituted benzaldehydes mediated by $Na_2S_2O_5$

Tab. 4. 2-aminobenzothiazole reaction with *p*-substituted benzaldehydes mediated by $Na_2S_2O_5$ – condition

entry	R	R^1	time (h)	t(°C)	Result
1.	- H	- OCH_3	2	120	-
2.	- CH3	- OCH_3	2	120	product detected
3.	- OCH3	- OCH_3	2	120	product detected
4.	- H	- OH	2	120	-
5.	- H	- NO_2	2	reflux	-
6.	- H	- OCH^3	2	reflux	-

In experiment no. 2. After recrystalisation with MeOH a yellow precipitate appeared. This precipitate should be the desired product, howewer one unusual extra-peak appearedin NMR spectra which could be in my opinion the peak of unsubstitutedbenzothiazole. Therefore deamination of our starting compound could be possibly the side reaction .In the case of exp no. 3. the product was detected in reaction mixture (no purification). In other cases there wasn´t even a trace of product in reaction mixture.

4.2.1.3. Procedure 3

Because of high residual solvent peak in NMR spectra (DMSO) in previous case was decided to use DCM/water extraction instead of diethylether/water. We also made more extractions than in the previous case. We assumed that presence of DMSO in final product decreased the resolution and peaks of our desired product would be sharper and easily detectable after changing extraction conditions. .In some cases I used DMF instead of DMSO to observe effect of solvent to the reaction.

The 6-substituted/unsubstituted 2-aminobenzothiazole and appropriate *para*-substituted benzaldehyde were dissolved in dry solvent (tab 3.). To the resulting mixture the sodium metabisulfite was added and the mixture stirred at various temperatures (tab 5.)

for various time (tab 5.). After cooling to the room temperature the water (40ml) was addend and the mixture was extracted 5-times with DCM (30ml). Organic layer was washed 3-times with water following washing with brine and subsequently dried over sodium sulphate and evaporated under reduced pressure. Yellow unpleasant-smelling oil was obtained.

Tab. 5. 2-aminobenzothiazole reaction with *p*-substituted benzaldehydes mediated by $Na_2S_2O_5$ – condition - 2

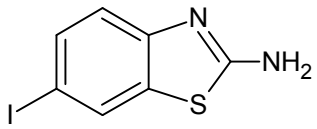
entry	R	R1	Time(h)	Solvent	t(C°)	Result
1	-OMe	-OMe	2	DMSO	120	product detected
2	-H	-OMe	2	DMF	120	-
3	-H	-OH	2	DMSO	120	-
4	-H	-OMe	8	DMSO	reflux	-
5	-H	$-NO_2$	2	DMSO	120	-
6	-H	$-CH_3$	2	DMF	120	-

4.2.2. Discussion

Both of above mentioned method seem not to be suitable for 2-phenylbenzothiazoles synthesis. The temperature did not increase yields and as also the solvent seemed to have no effect for forming benzothiazole moiety. However 6-substituted 2-aminobenzothiazoles have shown several possibilities to form our desired product in those conditions. Therefore only suggestion for future research is to perform the reaction with 6-substitution under different condition. In my opinion the only way worth it is remain condition showed in table 2 or 3 and treat the compounds with microwave irradiation or using *p*-TSA as the catalyst. Both previously used solvents are strong dipoles and therefore prospective solvents for microwave condition.

4.3. [^{18}]F – labelled 2-phenylbenzothiazole

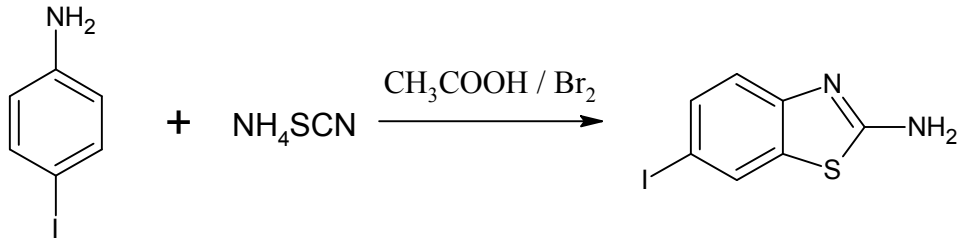
4.3.1 Synthesis of 2-amino-6-iodobenzothiazole



112

Figure.19. 2-amino-6-iodobenzothiazole

At first, it was decided to use probably the easiest way to synthesise **112** including one step reaction and rather easy purification with recrystallisation.



113 **114** **112**

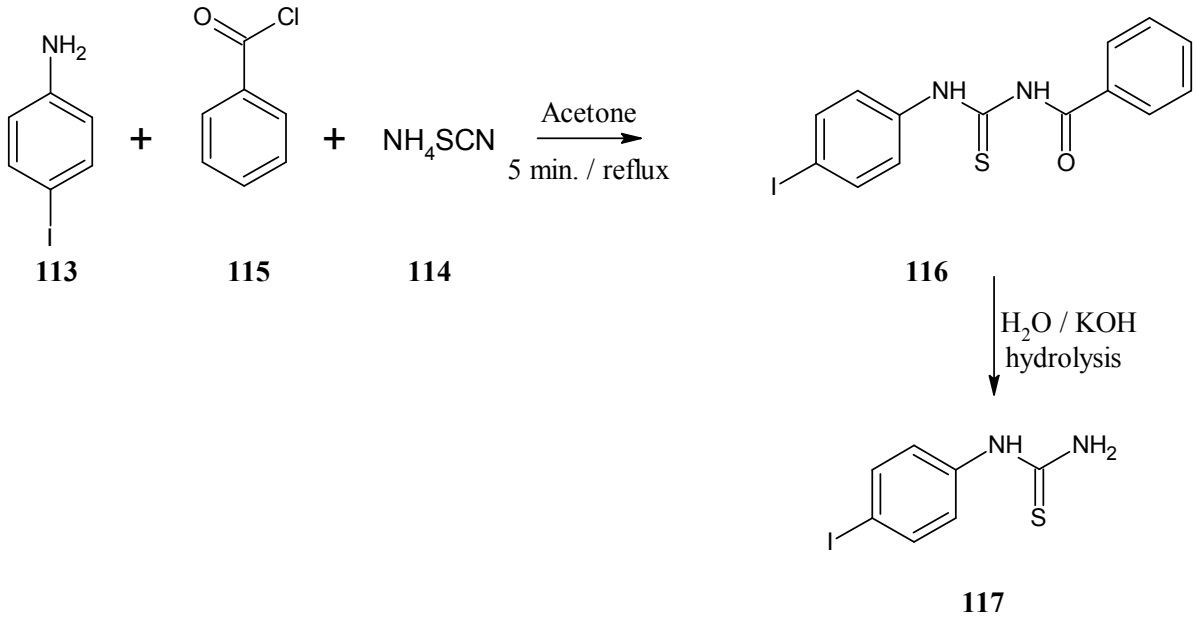
Scheme 31. Synthesis of 2-amino-6-iodobenzothiazole

Although highly reported in literature and supposed to give high yields [90,91,92] , the above – mentioned reaction ,Scheme 1, had given poor results .Even if we tried different conditions (as various temperature , prolonged reaction time , inert atmosphere etc…) , reaction mixture did not show traces of product in NMR spectra. The conditions are described in Table 6.

Table 6. 2-amino-6-iodobenzothiazole synthesis - conditions

entry	Iodoaniline eq.	NH_4SCN eq.	Br_2 eq.	solvent	temperature (°C)	time (h)	result
1.	1	1 ,1	1,1	CH_3COOH	10	1	-
2.	1	3	0,9	CH_3COOH	r.t.	1	-
3.	1	1 ,5	1	CH_3COOH	80	5	-
4.	1	3	1	DCM	r.t.	overnight	-
5.	1	3	1	Chloroform	Reflux	2	-

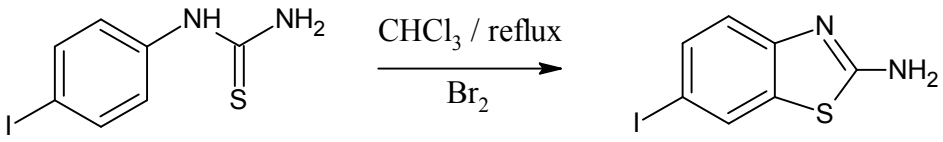
After unsuccessful reaction Scheme 31 it was decided to split this reaction into two reaction steps .At first to synthesise a substituted thiourea (Scheme 32.)[93]. This reaction gave high yields and no difficult purification was required, because the desired compound is not water-soluble .After alkalization, benzoic acid (as a by product) stayed in water in its water soluble form.



113 **115** **114** **116** **117**

Scheme 32. Synthesis of 1-(4iodophenyl)thiourea

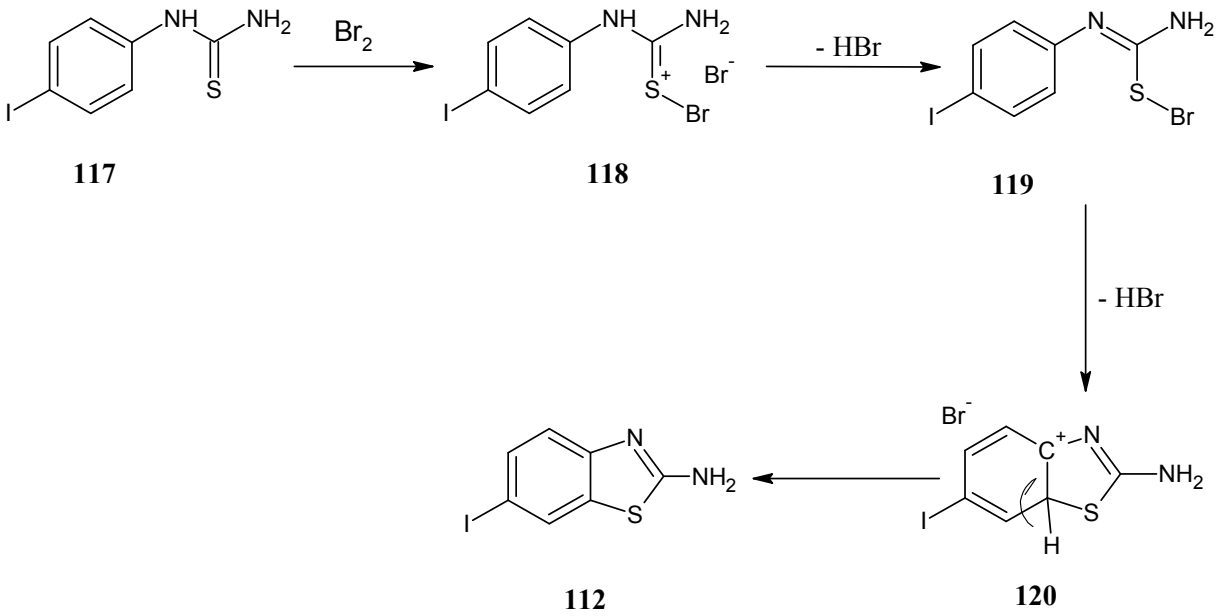
Subsequently the amino-substituted thiazole ring was created via bromide mediated cyclisation, Scheme 33.. Other useful cyclisation-mediating agent could be the elemental iodine which seems to be more advantageous because of its low health risk potential (it is a solid chemical and therefore is easily to work with)[60].



117 **117**

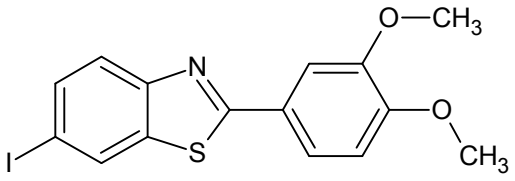
Scheme 33. Bromine mediated thioamide cyclisation

As shown in the reaction Scheme bellow (Scheme 34.) the crucial step is thought to be the sulfenylbromide, **118**, formation followed by elimination of HBr. After that the sulfenyl bromide attacks the electron rich aromatic ring, disrupts it and subsequently stabilizes itself by proton elimination [94]. Alkalization of reaction mixture could be a very helpful way to increase yields. Hydrobromic acid as by-product in reaction mixture could possibly peritonize basic nitrogen in the molecule and therefore increases water solubilityof product during organic extraction.



Scheme 34.Mechanism of reactionleadingto 2-amino-6-iodobenzothiazole.

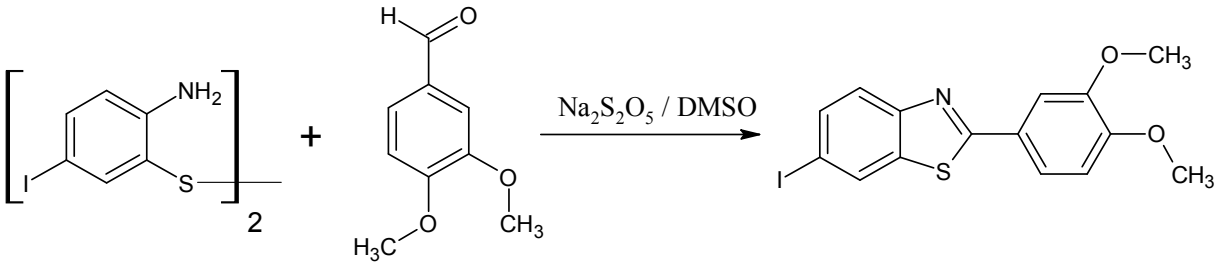
4.3.2. Synthesis of 6-iodo-2-(3,4-dimethoxyphenyl)benzothiazole



121

Figure 20. 6-iodo-2-(3,4-dimethoxyphenyl)benzothiazole

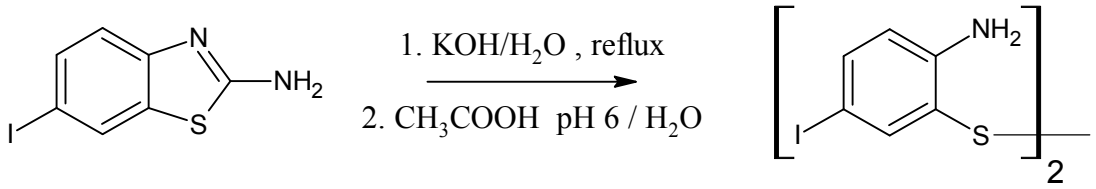
Firstly it was decided to prepare **121** using reaction of 6-iodosubstituted 2-aminothiophenol disulfide with appropriately substituted benzothiazole in presence of sodium metabisulfite as mild oxidant and in DMSO as a solvent [55] Scheme 35. It was decided to use 3,4-dimethoxybenzaldehyde as a starting compound because of methoxy group chemical stability and also because several 3´,4´-dimethoxy substituted 2-phenyl benzothiazole have been previously proved as compound with high anticancer and cancer *in vitro* ability (GW 610).



122 **123** **121**

Scheme 35. Synthesis of 2-(3,4-dimethoxyphenyl)-6-iodobenzothiazole.

Synthesis of 5-iodo substituted 2-aminothiophenol disulfide was made in accordance to published method [55]. The method includes hydrolytic cleavage of the thiazolering, mediated by potassium hydroxide (NaOH usually gave no or only poor results), followed by air oxidation Scheme 36:



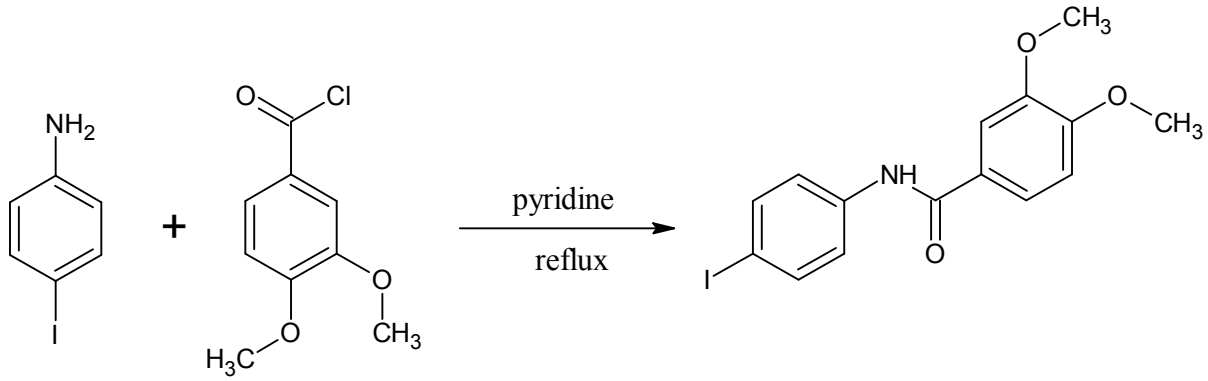
112 **122**

Scheme 36. Hydrolytic cleavage of thiazolering subsequently followed by air oxidation leading to bis(2-amino-5-iodophenyl)disulfide

Although the method was originally developed with halogen (F,Cl,Br) substituted benzothiazoles, the 6-iodo substituted compound gave unconvincing results. After air oxidation there was only slight trace of product in the reaction mixture. Because of that it was decided to try one of classical way to create **122**.

After unsuccessful reaction, Scheme 36, I made a choice to form the iodo-substituted dimethoxyphenylbenzothiazole via Jackobsen cyclisation, Scheme 37. There are more reaction steps required comparing with previously mentioned reaction. However it is

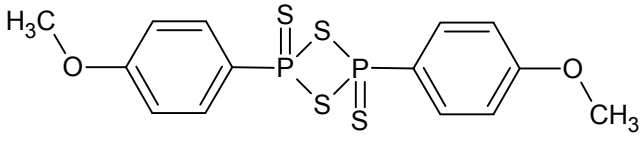
thought to be a classical way to benzothiazole ring formulation. First step is amide formation, Scheme 37. Amide was synthesised from *para*-iodoaniline and appropriate (3,4-dimethoxybenzoylchloride) acylchloride both commercially available in sufficient purity . Pyridine was used as solvent because of its high boiling point and proton scavenger ability. Both of those effects were supposed to increase yields.



113 **123** **124**

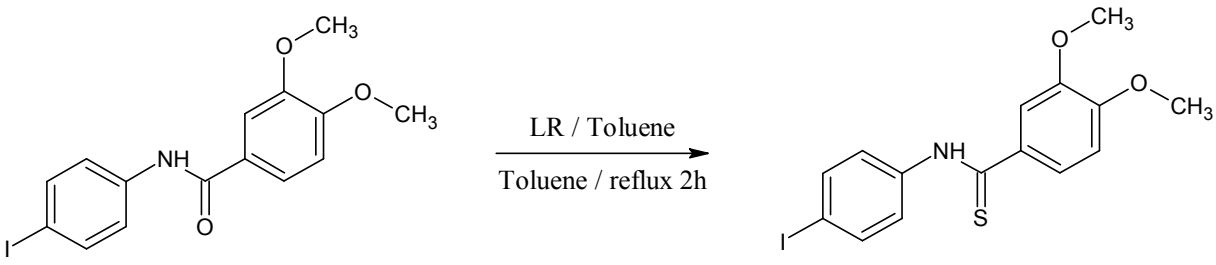
Scheme 37. Synthesis of *N*-(4-iodophenyl)-3,4-dimethoxybenzamide (124)

This short time reaction gave high yield and after simple purification afforded the desired product in high purity. Next step was replacing carbonyl oxygen with sulphur, Scheme 38.For this purpose Lawesson´s reagent,figure. 21., (LR) was chosen as the best option because of its high availability and relatively low price. LR is a solid chemical and therefore is easy to work with. However there are several limitation (disgusting smell and toxicity) of this compound.



125

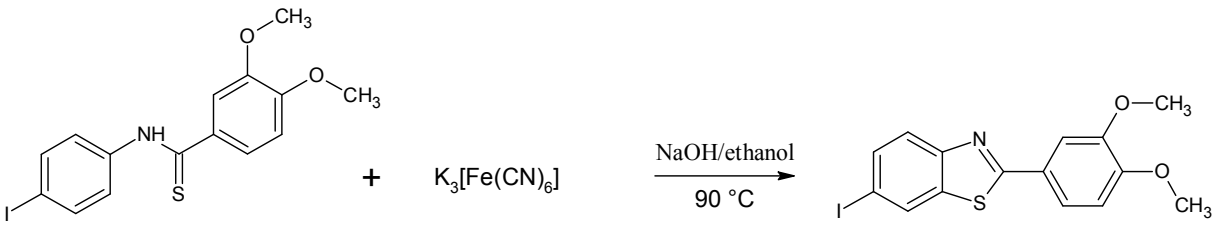
Figure.21. Lawesson´s reagent



124 **126**

Scheme 38.Thioamidepreparationvia Lawesson´s reagent mediated replacement of oxygen atom

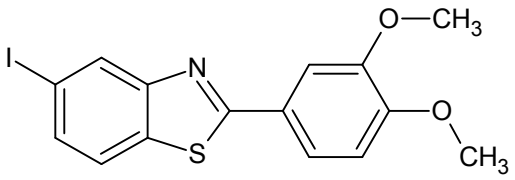
The following potassium ferricyanide mediated cycliszation, Scheme 39, gave unusually good results. The Yield after simply purification, including recrystalisation with methanol, was over 95%. However the dosing of the suspension of potassium ferricyanide with water and ethanol via syringe is difficult. It should to be added very slowly and the mixture must be kept in motion to avoid descending of particles. As explained further, this method is fully suitable for *para* substituted anilines as starting compound because there are no regioisomersresulting this reaction. Presence of slightly electron donating iodine atom is also advantageous for this reaction.



126 **127** **121**

Scheme 39.Jackobsen cycliszation affording 2-(3,4-dimethoxyphenyl)-6-iodobenzothiazole

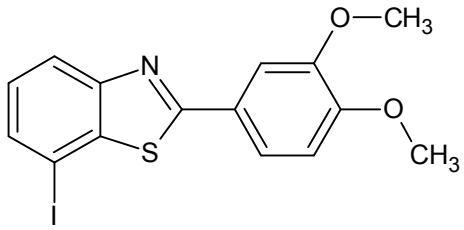
4.3.3. Synthesis of 2-(3,4-dimethoxy-phenyl)-5-iodobenzothiazole



128

Figure 22. 2-(3,4-dimethoxy-phenyl)-5-iodobenzothiazole

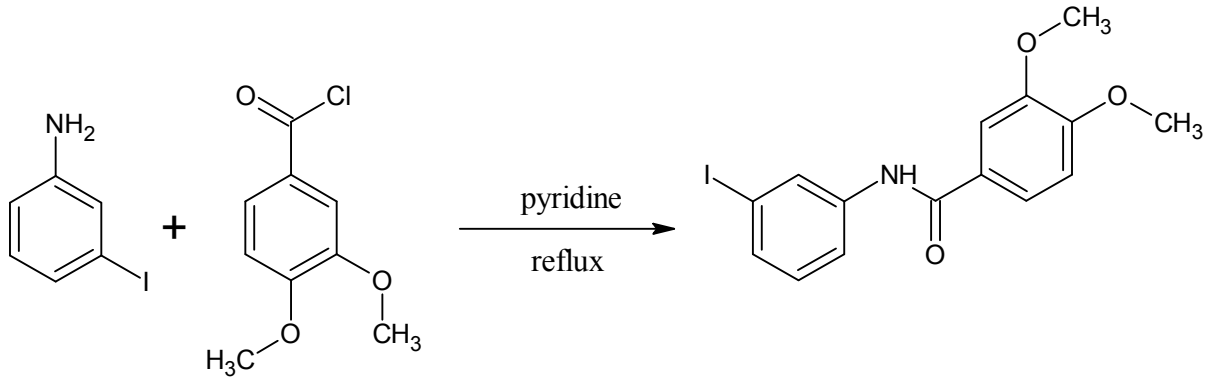
After success in previous reaction it was decided to synthesise **128** in the same way Scheme 39. The decision was made because of the price of starting compound (3-iodoaniline). It was presumed that presence of bulky iodine atom will prevent or at least decrease amount of unwanted 2-(3,4-dimethoxy)phenyl-7-iodobenzothiazole ,**129**.



129

Fig.23. 2-(3,4-dimethoxy-phenyl)-7-iodobenzothiazole (possible sideproduct of Jacobsoncyclisation)

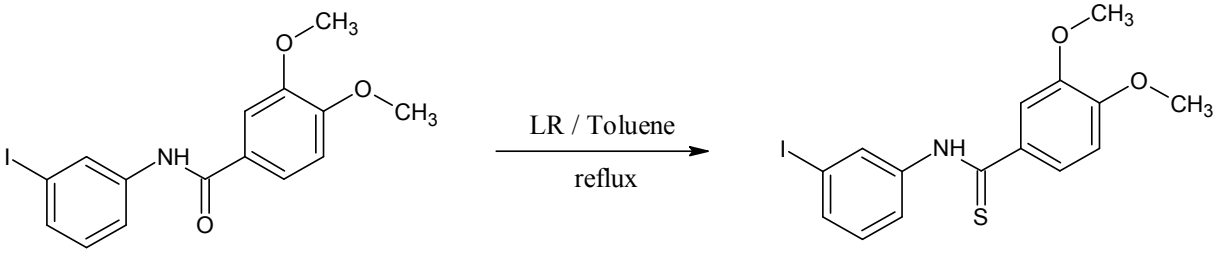
As previously, **123**, we started with (3,4-dimethoxybenzoylchloride) and appropriate iodoaniline to create amide bond, Scheme 40**.**



130 **123** **131**

Scheme 40. Synthesis of *N*-3´-iodophenyl-3,4-dimethoxybenzanilide

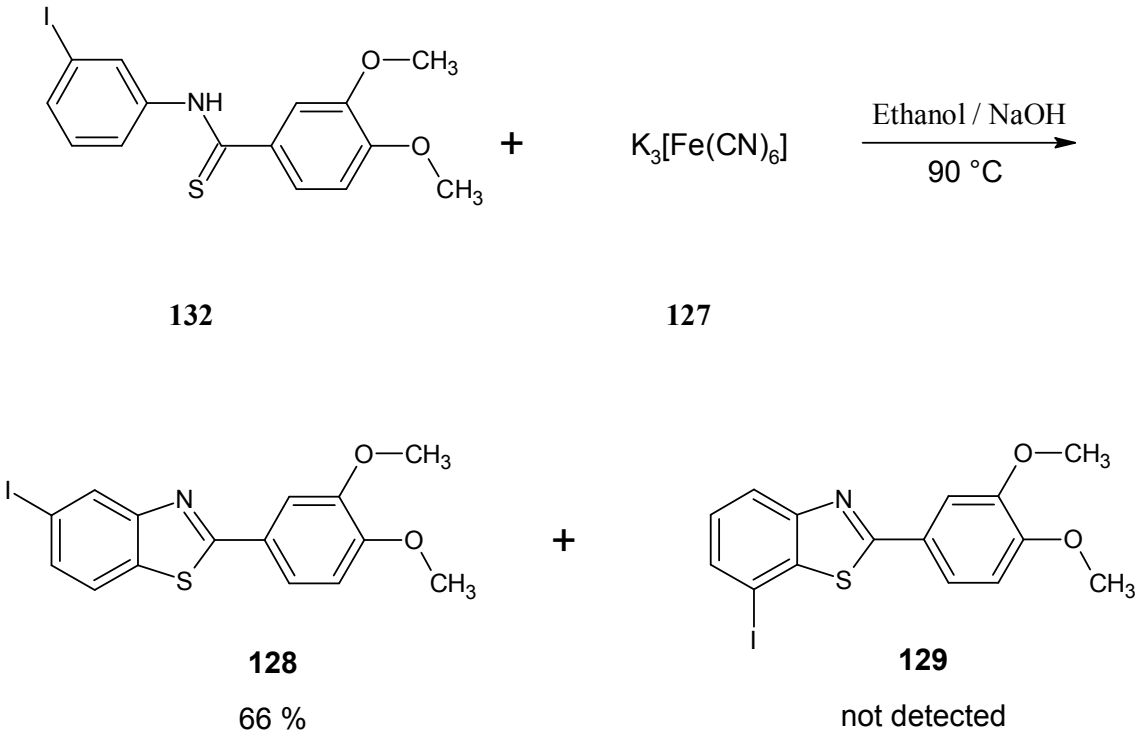
The reaction gave high yield but we could not avoid presence of starting compounds in reaction mixture because our desired compound was viscous oil. Therefore we had to change the purification method. We used organic/water ammonium hydroxide (15% solution) extraction. we got about 94% yield. In the next step (thioanilide formation) we used the same condition as for **126**synthesis, Scheme 41.



131 **132**

Scheme 41.Lawesson´s reagent mediated synthesis of N-3´-iodo-3,4-dimethoxythiobenzanilide

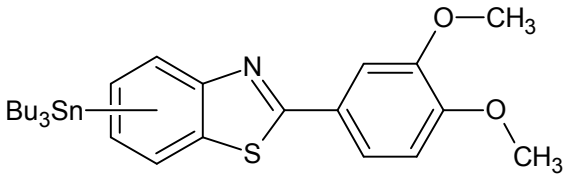
In comparison with **126** the result was disappointing. We got only 53% yield after purification including simple washing with methanol. It was thought it could be because of shielding the carbonyloxygen with iodine in meta-position on aromatic nucleus (dithiophosphineylide is relatively bulky). For future reaction I may suggest prolonged reaction time to get higher yields. The last step in synthesis of **128** was thioamide (Jacobson) cyclization Scheme 42**:**



Scheme 42.Jackobson cyclization of thioamide affording 5- iodosubstituted (3,4-dimethoxyphenyle)benzothiazole , 7-iodosubstituted (3,4-dimethoxyphenyle)benzothiazole was not detected

As apparent, there are two positions on aromatic nucleus available for cyclization. We presumed that on starting compound would be preferential because of the size of iodine atom preventing cyclisation in position 2. However I got **128** in 66 % of yield and the **129** was not identified in the NMR sample after recrystallisation with methanol. Therefore the foregoing method seems to be fully suited for **128** -synthesis.

4.3.4. Synthesis of 2-(3,4-dimethoxyphenyl)-6-tributylstanylbenzothiazole and 2-(3,4-dimethoxyphenyl)-5-tributylstanylbenzothiazole

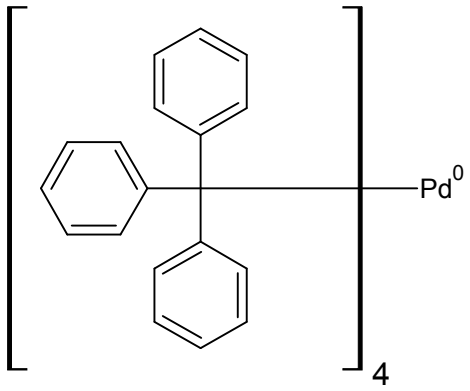


6 $SnBu_3$ = **133**

5 $SnBu_3$ = **134**

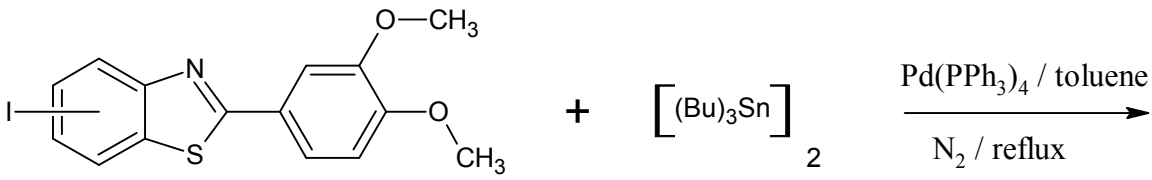
Figure. 24. 2-(3,4-dimethoxyphenyl)-6-tributylstanylbenzothiazole (133) and -(3,4-dimethoxyphenyl)-5-tributylstanylbenzothiazole

After successful reaction resulting to compound **121** and **128** we finally got to the synthesis of a precursor of [^{18}F]-labelled derivatives of GW 610 **133** and **134**. .**121** and **128** were treated with bis(tributyltin) **136** in presence of tetrakis(triphenylphosphine)palladium **134** as a catalyst .This reaction was has been welldescribed and promised to give from moderate to good yields [83,95] .The price of palladium catalyst and toxicity of organotin compound were the only limitations of this reaction. Mechanism of this reaction is shown bellow, Scheme 43.



135

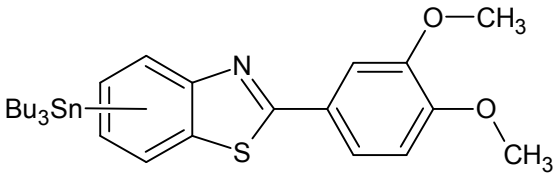
Figure 25. Tetrakis(triphenylphosphine)palladium(0), Pd(PPh3)4



6 I= **121**

5 I= **128**

136



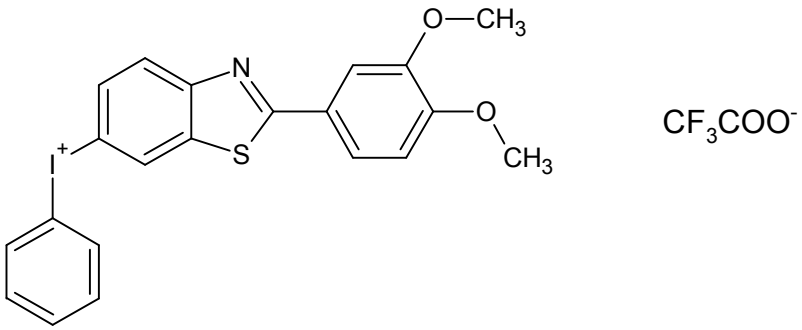
6 $SnBu_3$ = **133**

5 $SnBu_3$ = **134**

Scheme 43.Pd(PPh3)4 catalyzed synthesis of 133 and 134.

After extraction and purification with column chromatography the reaction gave moderate yield 53% of pure product for 133 and 31% for 134.This product, as shown previously, could be easily modified with radio-labelled fluorine gas to give related [^{18}F]-derivatives (see chapter 3).

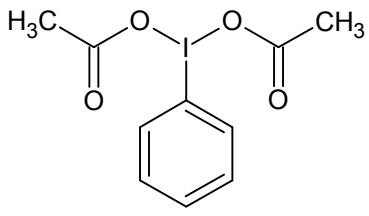
4.3.5. Synthesis of 2-(3´4´-dimethoxyphenyl)-6-phenyliodoniumbenzothiazol trifluoroacetate



137

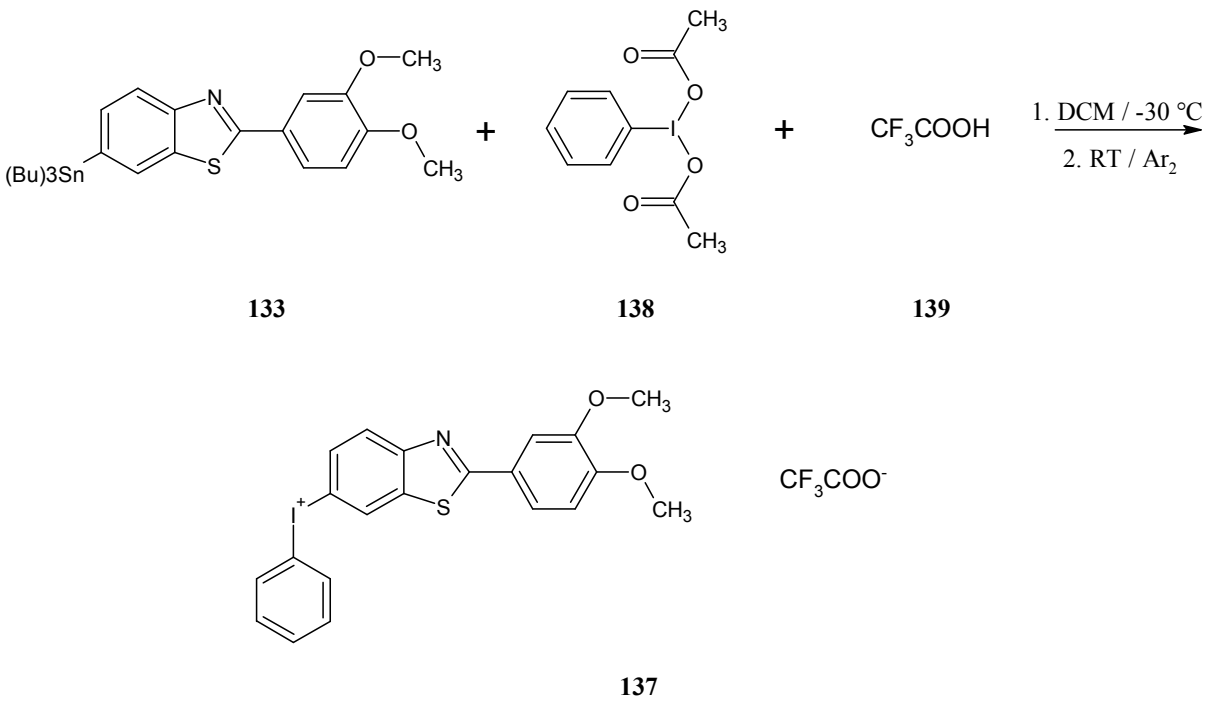
Figure 26. 2-(3´4´-dimethoxyphenyl)-6-phenyliodoniumbenzothiazol trifluoracetate

The structure above was one of most promising precursors for [^{18}]F labelling due to its frequent references about aryliodonium labelling (see chapter 3) and its high reactivity .Another advantage of this compound as a precursor is its high reactivity supposed to result in rapid reaction time(half live of [^{18}]F is relatively short) and increase the yield. I started with organotin compound **133** , (diacetoxyiodo)benzene, **138,** (commercialy available from Sigma-Aldrich) and trifluoracetic acid using dichlormethane as a solvent. We used fresh anhydrous solvent, absolutely dry apparatus and argon atmosphere to avoid possible side products because of reactivity of desired compound.



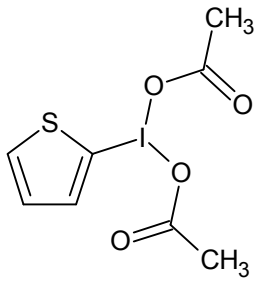
138

Figure 27. (Diacetoxyiodo)benzene



Scheme 44.Preparationof diaryliodoniumtrifluoracetate

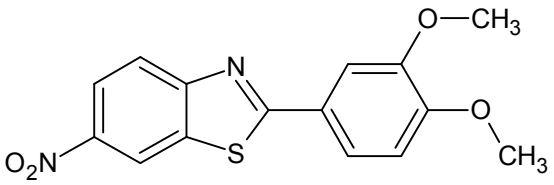
Even all condition was kept as mentioned in literature [83] I still was unable to get the product. I used only very small amount of starting tin compound so therefore maybe I destroyed the product during the purification (presence of water in MeOH I used for recrystallization, high temperature of methanol). For future experiments we suggest using larger amount of starting compound, prolonged reaction time and it would maybe also be useful to use another diacetoxyiodo compounds as a starting material. I especially suggested the Koser´s reagent due to its high availability in high purity. Other possibility could also be the diacetoxyiodoheteroarenes due to high electron density which could positively affect the reaction (increasing yields and shorten the reaction time). I also tried to synthesise one of them (diacetoxydoiodo-2-thiophene). Unfortunately I did not succeed even if I tried to synthesise it for several times underdifferent condition mentioned in the literature [83].



140

Figure 28.2-(Diacetoxydiiodo)-thiophene

4.3.6. Synthesis of -(3,4-dimethoxyphenyl)-6-nitrobenzothiazole

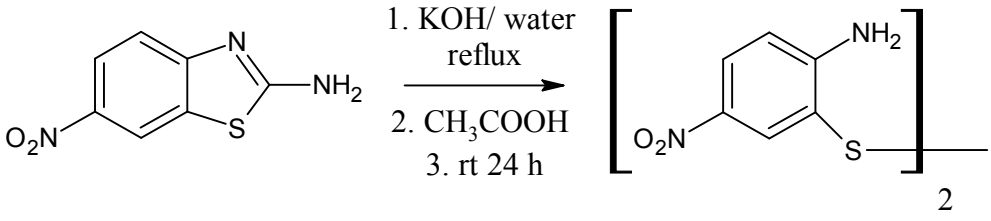


141

Figure. 29. 2-(3,4-dimethoxyphenyl)-6-nitrobenzothiazole

The structure above, **141**, is one of possible precursor of [^{18}]F labeled 2-(3,4-dimethoxyphenyl)-6-fluorobenzothiazole. There are many of literature sources suggesting replacement nitro group with a fluorine ion available. Although most of them are dealing with electron withdrawing group on aromatic nucleus in an appropriate position (such as aldehyde or another nitro group in *o* or *p* position) some authors also suggests that unsubstituted or slightly electron-donating substituted compounds may give some results (see chapter 4). I therefore assumed that slightly electron withdrawing effect of the thiazole ring (due to negative inductive effect of N atom) could positively affect the nucleophilic substitution. I assumed that some harsh reaction condition (high temperature, polar high boiling solvent) could also increase the result of this reaction. We decided first to try again the substituted aminodisulfide reaction. We first tried to

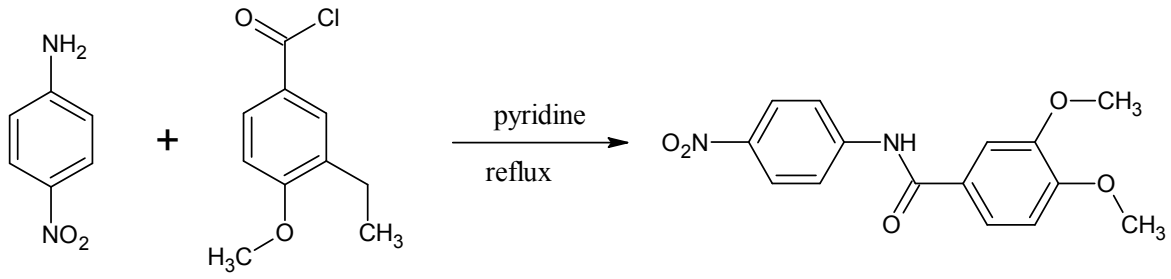
prepare 2-amino-5-nitrodisulfide **143** following the Weekes *et al.* procedure Scheme 45 [55].



142 **143**

Scheme 45. Potassium hydroxide mediated cleavage of thiazolering followed by air oxidation forming bis(2-amino-5-nitrophenyl)disulfide 143

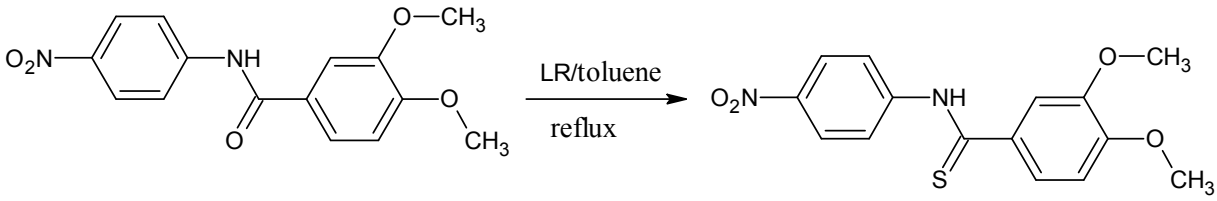
Unfortunately I haven´t got at least a trace of product in NMR spectra, therefore I decided to try some other method. It seemed that the Jacobson cyclization would be useful. I established the conditions before and in case of the iodine substituted fenylbenzothiazoleit gave excellent yields. First step is again the amide formulation in pyridine as solvent, Scheme 46 . I assumed that the reaction will give lower yield than 3,4-dimethoxy-*N*-(4-iodophenyl)benzamide due to electro-withdrawing effect of nitro group in *p*- position. To overcome this obstacle it was decided to use prolonged reaction time.



144 **123** **145**

Scheme 46.Preparation of 3´-nitrophenyl-3,4-dimethoxybenzanilide

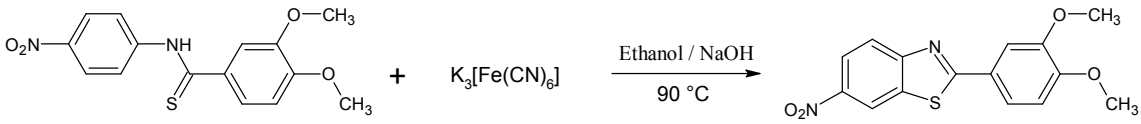
After simple purification (washing with water and methanol) I got pure product in excellent(considering the presence of *p*-substitution with nitro group) yield of 93%. After that I could proceed to the next step , the thiocarbamide synthesis Scheme 47.As well as in the previously mentioned iodosubstituted compound, I used LR as thiolation agent. Because of the presence of nitro group in *par*a position of the molecule which could possibly resulting to decrease the reactivity it was decided to use prolonged reaction time.



145 **146**

Scheme 47.Lawesson´s reagent mediated synthesis of 3´-nitrophenyl-3,4-dimethoxythiobenzanilide

After simple purification (recrystallization with MeOH) we got the product in sufficient purity and yield 80%. Final step of this reaction was Jacobson cyclisation Scheme 48.



146 **127** **147**

Scheme 48.Jackobson cyclization of thioamide affording 2-(3,4-dimethoxyphenyl)-6-nitrobenzothiazole

After ether/water extraction followed by recrystallization with methanol we got the desired product in moderate yield (53%). The advantage of this particular reaction is that there should be no isomers of nitro substituted 2-(3,4-dimethoxy)benzothiazole given. It is because of the fact that the *meta* positions in *p*-nitroaniline are chemical equivalent and therefore both equally available for cyclisation. The low yield (in comparison with **121** and **128** is the result of presence of deactivating nitro group in the

molecule. **147** is useful for both fluorination and preparing another useful fluorination precursor

4.3.7. Discussion

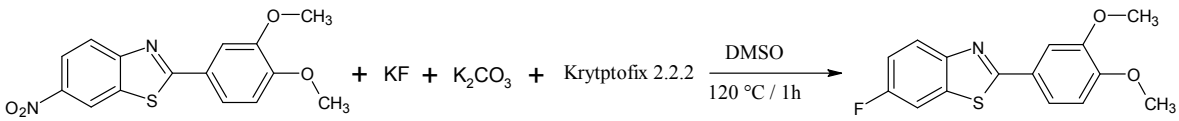
One precursor of [^{18}F]-G 610 for radiolabelling with [^{18}F]-F gas has been synthesised, **134**,. Apart from that **134** can be converted into related iodonium salt. For this purpose I suggest using Koser´s reagent instead of (diacetoxyiodo)benzene. My suggestion for purification of product is to use either absolutely (fresh) anhydrous solvent or to use centrifugation instead of crystallization.

Two precursors of 6-isomer of [^{18}F]-GW 610 have been synthesised, **133** and, **147**. **133** can be used for radiolabelling with [^{18}F]-F gas or can be converted into related iodonium salt. My suggestion for future synthesis and purification are the same as in previous case. **147** is perspective precursor for direct nucleophilic radiolabelling with F^- in high-boiling solvent in presence of Kryptofix 2.2.2

4.4. Direct nucleophilic substitution of nitro group using F^- as nucleophile.

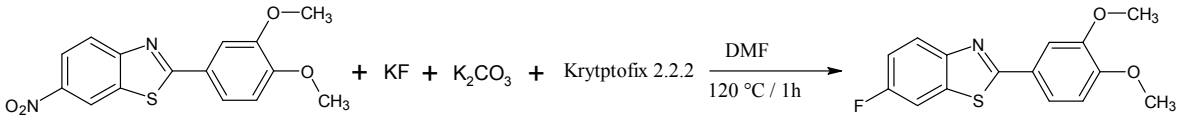
Getting inspiration from methods reported in chapter 4 I performed two direct nucleophilic substitution using F^- as nucleophile to find optimal condition for future labelling with radioactive fluorine in a laboratory with special radiochemistry equipment. I decided to try the nitro derivative (NO_2 as potential leaving group) using DMF and DMSO as high-boiling solvents..

I made these reactions with either DMSO, Scheme 48.or DMF, Scheme 49.as solvent, kryptofix 2.2.2., **92**, as the phase transfer catalyst and K_2CO_3 as base, **149**,. As the fluorination agent was chosen KF, **148**, due to the K^+ size. Because it is relatively small, it was thought that it could lead to the higher binding ability to Kryptofix, and therefore increased the presence of F^- in reaction mixture.



147 **148** **149** **92** **150**

Scheme 48.Nucleophilic substitution using fluorine anion as nucleophile, kryptofix 2.2.2 as phase transfer catalysis and DMSO as solvent leading to 2-(3,4-dimethoxyphenyl)-6-fluorobenzothiazole



147 **148** **149** **92** **150**

Scheme 49.Nucleophilic substitution using fluorine anion as nucleophile ,kryptofix 2.2.2 as phase transfer catalysis and DMF as solvent leading to 2-(3,4-dimethoxyphenyl)-6-fluorobenzothiazole

In the first case, we used fresh (therefore absolutely anhydrous)DMSO as solvent and treated the reaction mixture at 120°C under argon atmosphere. After 1 hour we cooled the mixture and poured it into water. We extracted it with DCM for 3 times to avoid presence of DMSO and after washing with brine and drying with sodium sulfate we evaporated the solvent. On TLC there was a new spot higher than the spot of the nitro-compound (estimated log Pis for 147 - 4,23 and for 150 – 4,55 (using ACD/Log Pfor calculation). The new spot was also blue fluorescing under long wave UV (365nm). In my opinion in could be the desired fluoroderivate , because of its both higher lipofilicity than the nitro compound, and different conjugation of π electrons caused by leaving nitro group (starting compound fluorescent green under long wave UV- 365nm). I therefore tried to isolate the compound via preparative TLC with increased hydrophility of mobile phase (for the first time with pure hexane, for t next two times with mixture of hexane and ethylacetate 10:1 and for the last time with hexane and ethylacetate 5:1). Although I successfully isolated what was supposed to be our desired product, in NMR spectra a trace of **150** was visible neither in ^{19}F nor in ^{1}H spectra. It could be also because of very small amount of desired compound isolated from the reaction mixture

as the basal line on ^{1}H spectra was almost straight and except solvent peak there was no other peak visible.

In the second case we used dry DMF instead of DMSO and observed whether the replacement of solvent will give some product .We decided to use the same condition as in the previous case .The reaction was monitored by TLC but after 1 hour there was no spot above the nitro- compound spot visible. After 1 hour we used the same purification workup as in the previous case. In this case we decided to use only the ^{19}F NMR experiment for checking out the presence of our desired compound .However there was not significant peak in the spectra.

4.4.1. Discussion

In my opinion it will certainly be advantageous to carry on the experiment with DMSO, and maybe to change its conditions (microwave irradiation). Using MeCN as a solvent could be advantageous as well. Using preparative HPLC for isolating the product would be better way to get sufficient amount of the product.

Chapter 5

5. Experimental

5.1. General Experimental Conditions

5.1.1. Solvents and Reagents

The following anhydrous solvents were bought from Sigma-Aldrich: Chloroform ($CHCl_3$), Dimethylsulfoxide (DMSO), *N*,*N*-dimethylformamide (DMF). All other reagents commercially available in sufficient purity from Sigma-Aldrich were used without further purification.

5.1.2. Thin Layer Chromatography (TLC)

Precoated, aluminium backed plates (60 F_{254}, 0.2mm thickness, Merck) were visualized under both short and long wave ultraviolet light (254nm and 366nm). Mixture of hexane and ethylacetate in 8:2 or 7:3 of rate was used as mobile phase for TLC chromatography

5.1.3. Column Chromatography (CC)

Column chromatography processes were carried out using silica gel supplied by Fischer (60A, 35–70μm). Mixture of hexane and ethylacetate in 9:1 of rate was used as mobile phase for column chromatography

5.1.4. Nuclear Magnetic Resonance (NMR)

^{1}H-NMR (500 MHz), ^{13}C-NMR (125 MHz) and ^{19}F-NMR (471 MHz) were recorded on a Bruker Avance 500MHz spectrometer at 25°C. Spectra were calibrated to the residual signal of the deuterated solvent used. Chemical shifts are given in parts per million (ppm) and coupling constants (*J*) in Hertz.

The following abbreviations are used in the assignment of NMR signals; s (singlet), d (doublet), t (triplet), m (multiplet), dd (doublet of doublets), dt (doublet of triplets).

5.1.5. Melting Points

Melting points were measured on a Griffen melting point apparatus.

5.2. General procedures

5.2.1. General procedure A.: Polymer supported synthesis

The appropriate benzaldehyde (0.98 mol/eq), polymer-bound triphenylphosphine (0.98 mol/eq) and *p*-toluensulfonic acid(0.19 mol/eq) were added to 2-aminothiophenol disulfide in mixture of toluene and DMF(1eq/1eq) and refluxed . The reaction was monitored by TLC. After the reaction was completed, the mixture was cooled, filtered through Celite® and the polymer washed with excess of either methanol or acetone. The filtrate was than evaporated under reduced pressure and water has been subsequently added. Precipitate was than collected, washed well with excess of water and dried. Purity of desired product was checked by TLC and recrystalization with methanol was performed if needed.

Scheme 27

5.2.2. General procedure B.: Amide synthesis

The solution of 4,4-dimethoxybenzoylchloride(1mol/eq) in pyridine was slowly added to the well stirred solution of appropriate substituted aniline(1mol/eq) in pyridine. The resulting mixture was refluxed for next 1hour. After cooling to the r. t., the mixture was poured into water. Resulting precipitate was collected and washed with water and methanol to give pure product.

Scheme 38, Scheme 40, Scheme 46

5.2.3. General procedure C.:Thioamide synthesis.

The appropriate amide (2 mol/eq) was dissolved in toluene and heated to 60°C. To this solution the Lawesson´s reagent (1 mol/eq) was added at once. Resulting mixture was refluxed until the amide spot on the TLC disappeared (approx. 2 h). Toluene evaporated

under reduced pressure and the resulting mixture let stayed overnight in a fumecupboard. After that, ethanol was added. Mixture was filtered and the resulting precipitate recrystallized from hot ethanol to give pure product.

Scheme 38, Scheme 41, Scheme 47

5.2.4. General procedure D.: Thioamide cyclization.

The appropriate thioamide (1 mol/eq) was suspended in small amount of 96% ethanol. To this suspension 30% water solution of NaOH was added. Resulting mixture was added dropwise via syringe to vigorously stirred and heated (90°C) water solution (20%) of potassium ferricyanide (4mol/eq). Reaction stirred and heated (90°C) and monitored by TLC until complete. Cooled to RT and filtrated. Precipitate was collected and washed with large excess of water. Recrystalization with methanol gave product in sufficient yields.

Scheme 39, Scheme 42, Scheme 48

5.2.5. General procedure E.: Tributylstannylbenzothiazole synthesis

To a stirred solution of appropriate iodosubstituted benzothiazole (1 mol/eq) in anhydrous toluene was added under argone atmosphere bis(tributyltin) (2 mol/eq) was added in one portion. To this mixture,tetrakis(triphenylphosphine)palladium(0) in catalytic amount($8{,}6 \cdot 10^{7}$ mol/eq) was added. Reaction mixture was allowed to reflux until complete (monitored by TLC) under argon atmosphere. After finishing the mixture was cooled to r t and the solvent removed using slight stream of nitrogen. The mixture was than purified by column chromatography (silica gel / hexane:ethylacetate in 9:1 of ratio) to afford product in sufficient purity and yield.

Scheme 43

5.2.6. General procedure F.: Phenylthiourea preparation

Benzoylchloride (1mol/eq) was added dropwise tothe suspension of ammonium thiocyanate (1mol/eq) in acetone. The mixture was then stirred and refluxed for 5 minutes and subsequently cooled to r t .To this solution iodoaniline (0,91mol/eq) dissolved in acetone was added and the resulting mixture stirred and refluxed until the reaction was complete (monitored by TLC) . Then the mixture was cooled to the r t and 5ml of 10% solution of NaOH in water was added and the mixture refluxed for next 2 hours. After that the condenser was removed, and the residual acetone allowed to evaporate. Solution was basified with 10% of water ammonium solution to approximately pH 11.Precipitate was collected, washed with large excess of water followed by diethyl ether.

Scheme 32

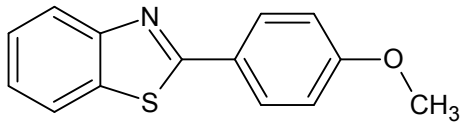
5.2.7. General procedure G.: formulation 2-aminobenzothiazole via phenylthiourea cyclisation

To vigorously stirred solution of appropriate phenylthiourea (1 mol/eq) in chloroform the solution of bromine (1 mol/eq) in chloroform was added dropwise over 20 minutes. Resulting mixture was allowed stirring at r t and subsequently stirred at reflux for next 1 hour. Mixture was then cooled to r t, filtered and the solid washed with chloroform. Resulting precipitate was suspended in 30% KOH solution. This mixture was extracted 3 times with ethyl acetate, collected organic layers washed with brine and dried over sodium sulfate. Ethylacetate was evaporated. Recrystallization with chloroform gave pure product in a good yield.

Scheme 33

5.3. Reactions and data

2-(4-methoxyphenyl)-1,3-benzothiazole - 101

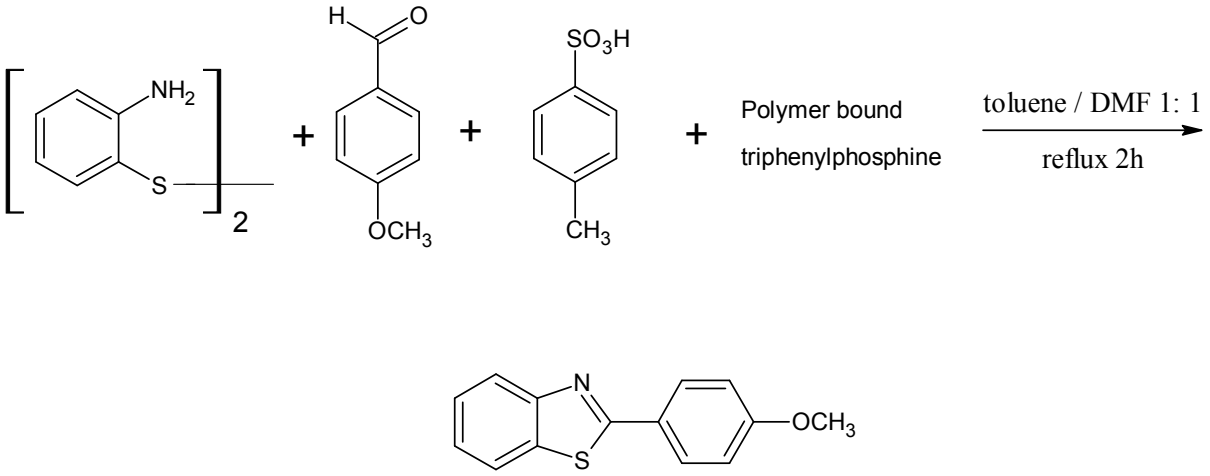


$C_{14}H_{11}NOS$

Mol.Wt.: 241.308

M.p.- 122-125°C

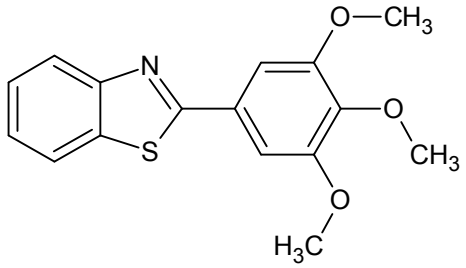
Prepared according to procedure A from para-anisealdehyde (0.18 ml), polymer-bound triphenylphosphine (0.5 g) and p-toluensulfonic acid (0.055 g) were added to 2-aminothiophenol disulfide (0.38 g) in mixture of toluene and DMF 1:1 (5ml) as white solid in 60% yield.



^{1}H-NMR (500MHz, $CDCl_3$) δ (ppm) 7.97-7.94 (m, 3H, H-2', H-6', H-4), 7.79 (d, J=7.5Hz, 1H, H-7), 7.39 (t, J=7.5Hz, 1H, H-5), 7.27 (t, J=7.5Hz, 1H, H-6), 6.93-6.92 (m, 2H, H-3', H-5'), 3.81 (s, 3H, OMe)

δ^{13}C (125MHz, $CDCl_3$) δ (ppm) 167.73, 154.06, 153.57, 140.70, 135.03, 129.02, 126.65, 125.50, 123.25, 122.40, 105.02, 30.85

2-(3,4,5-trimethoxyphenyl)-1,3-benzothiazole- 102

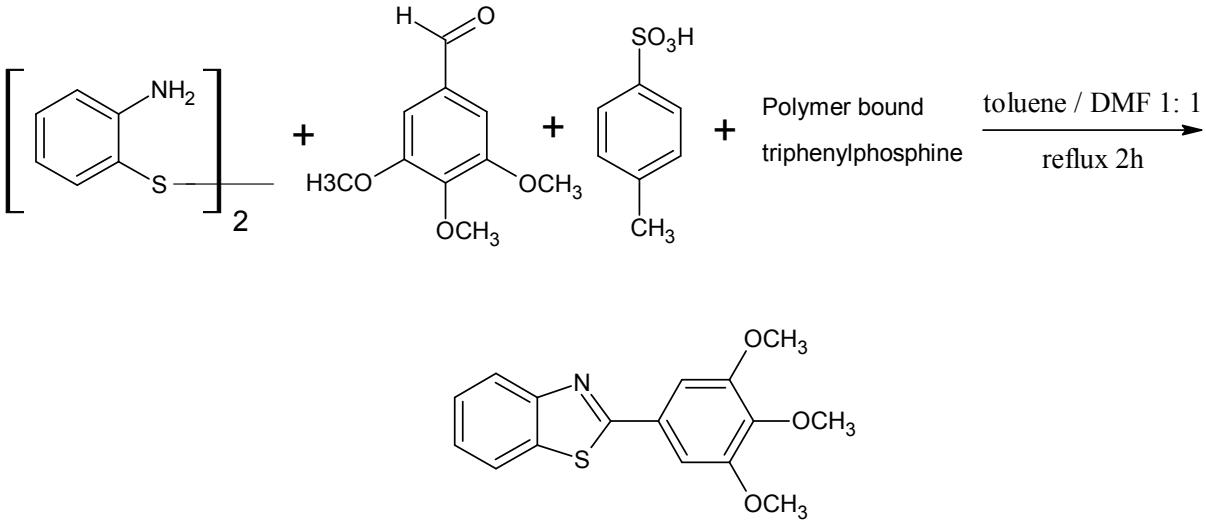


$C_{16}H_{15}NO_3S$

Mol. Wt.: 301.360

M.p. – 142-145°C

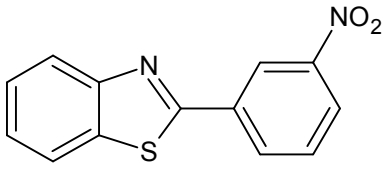
Prepared according to procedure A from 3,4,5-trimethoxybenzaldehyde (0.29 g), polymer- bound triphenylphosphine (0.5 g), p-toluensulfonic acid(0.055 g) and 2-aminothiophenol disulfide (0.38 g) in mixture of toluene and DMF 1:1 (5ml) and refluxed for 2 hours as yellow solid in 71% yield.



^{1}H NMR (d_6-DMSO) d 8.14 (1H, d, J = 8.0 Hz, H-4), 8.07 (1H, d, J = 8.0 Hz, H-7), 7.51 (1H, dt, J = 1.5, 8.0 Hz, H-5), 7.46 (1H, dt, J = 1.5, 8.0 Hz, H-6), 7.34 (2H, s, H-2', H-6'), 3.92 (6H, s, OMe), 3.76 (3H, s, OMe)

^{13}C-NMR (126MHz, d_6-DMSO) δ (ppm) 167.09, 153.44, 153.36, 140.25, 134.52, 128.27, 126.61, 125.38, 125.70, 122.19, 104.47, 60.20, 56.11.

2-(3-nitrophenyl)-1,3-benzothiazole- 103

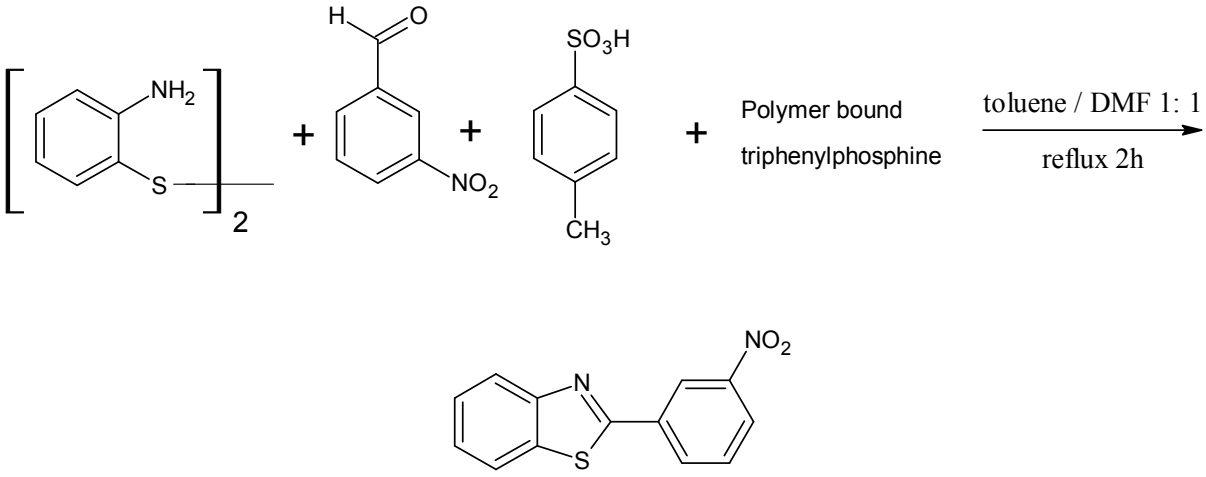


$C_{13}H_8N_2O_2S$

Mol. Wt.: 256.279

M.p. – 186-188°C

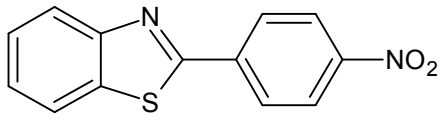
Prepared according to procedure A from 3-nitrobenzaldehyde (0.23 g), polymer- bound triphenylphosphine (0.5 g) , p-toluensulfonic acid(0.055 g) and 2-aminothiophenol disulfide (0.38 g) in mixture of toluene and DMF 1:1 (5ml) using acetone for Celite washing as brown solid in 39% yield.



^{1}H NMR (d_6-DMSO) d□8.84 (1H, t, *J* = 1.5 Hz, H-2'), 8.52 (1H, m, Ar-H), 8.42 (1H, m, Ar-H), 8.22 (1H, d, *J* =7.5 Hz, H-7), 8.16 (1H, d, *J* = 7.5 Hz, H-4), 7.88 (1H, t, *J* = 8.0 Hz, H-5'), 7.61 (1H, dt, *J* = 1.5, 7.5 Hz, H-6), 7.55 (1H, dt, *J* = 1.0, 7.5 Hz, H-5).

^{13}C-NMR (126MHz, d_6-DMSO) δ (ppm) 164.83, 153.27, 148.40, 134.72, 134.16, 133.42, 131.21, 127.02, 126.17, 125.57, 123.29, 122.61, 121.14.

2-(4-nitrophenyl)-1,3-benzothiazole- 104

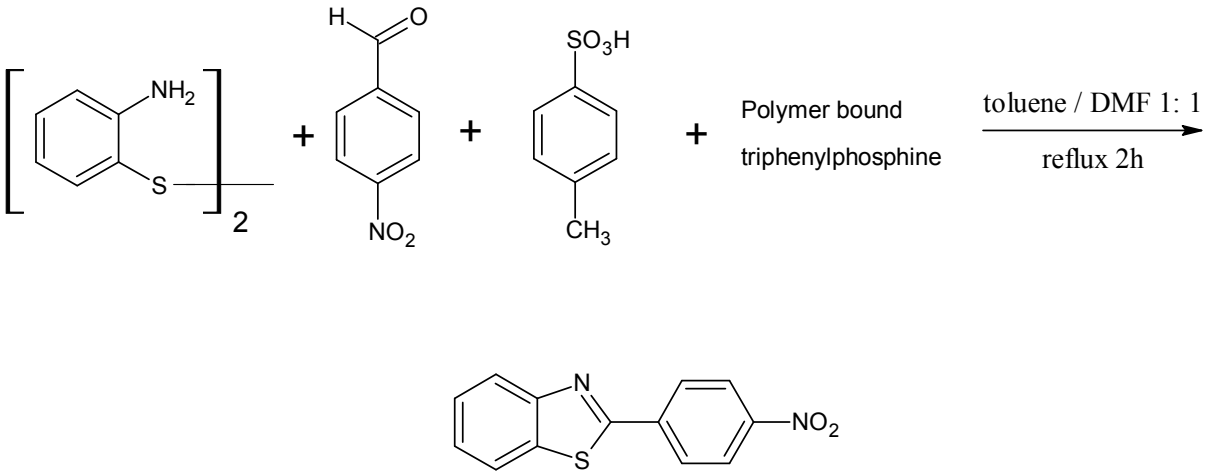


$C_{13}H_8N_2O_2S$

Mol. Wt.: 256.279

M.p. – 230-232°C

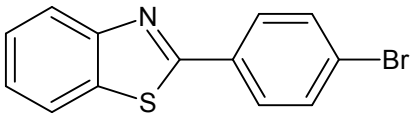
Prepared according to procedure A from 4-nitrobenzahldehyde (0.25 g), polymer-bound triphenylphosphine (0.5 g), p-toluensulfonic acid(0.055 g) and 2-aminothiophenol disulfide (0.38 g) in mixture of toluene and DMF 1:1 (5ml) using acetone for Celite washing as brown solid in 61% yield.



^{1}H NMR ($CDCl_3$) d□8.38 (2H, d, *J* = 8.5 Hz, H-3', H-5'), 8.30 (2H, d, *J* = 8.5 Hz, H-2', H-6'), 8.15 (1H, d, *J* = 8.0 Hz, H-4), 7.98 (1H, d, *J* = 8.0 Hz, H-7), 7.58 (1H, dt, *J* = 1.5, 8.0 Hz, H-5), 7.49 (1H, dt, *J* = 1.5, 8.0 Hz, H-6).

^{13}C-NMR (126MHz, $CDCl_3$) δ (ppm) 164.83, 154.11, 149.10, 139.19, 135.53, 128.24, 126.97, 126.23, 124.31, 123.92, 121.84

2-(4-bromophenyl)-1,3-benzothiazole- 105

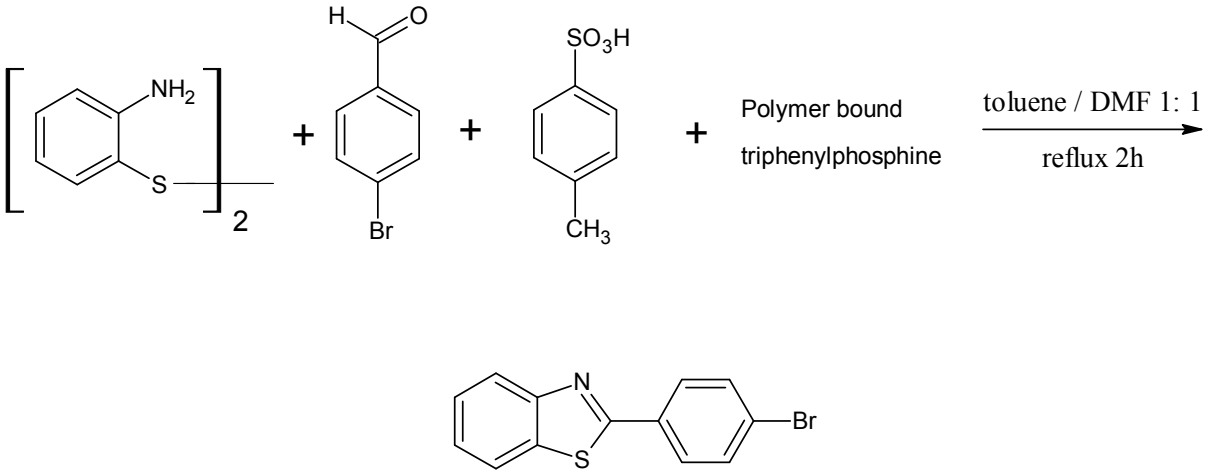


$C_{13}H_8BrNS$

Mol. Wt.: 290.178

M.p. – 127-129°C

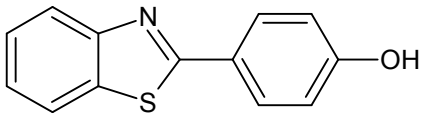
Prepared according to procedure A from 4-bromobenzaldehyde (0.278 g), polymer-bound triphenylphosphine (0.5 g) , p-toluensulfonic acid(0.055 g) and 2-aminothiophenol disulfide (0.38 g) in mixture of toluene and DMF 1:1 (5ml) as pink solid in 71% yield.



^{1}H NMR ($CDCl_3$) d□8.10 (1H, d, *J* =8.0 Hz, H-4), 7.99 (2H, d, *J* = 7.5 Hz, H-3', H-5'), 7.93 (1H, d, *J* = 8.0 Hz, H-7), 7.65 (2H, d, *J* = 7.5Hz, H-2', H-6'), 7.53 (1H, t, J =8.0 Hz, H-6), 7.43 (1H, t, J = 8.0 Hz, H-5).

^{13}C-NMR (126MHz, $CDCl_3$) δ (ppm) 166.69, 154.11, 135.07, 132.59, 132.25, 128.92, 126.51, 125.46, 125.42, 123.35, 121.67

2-(4-hydroxyphenyl)-1,3-benzothiazole- 106

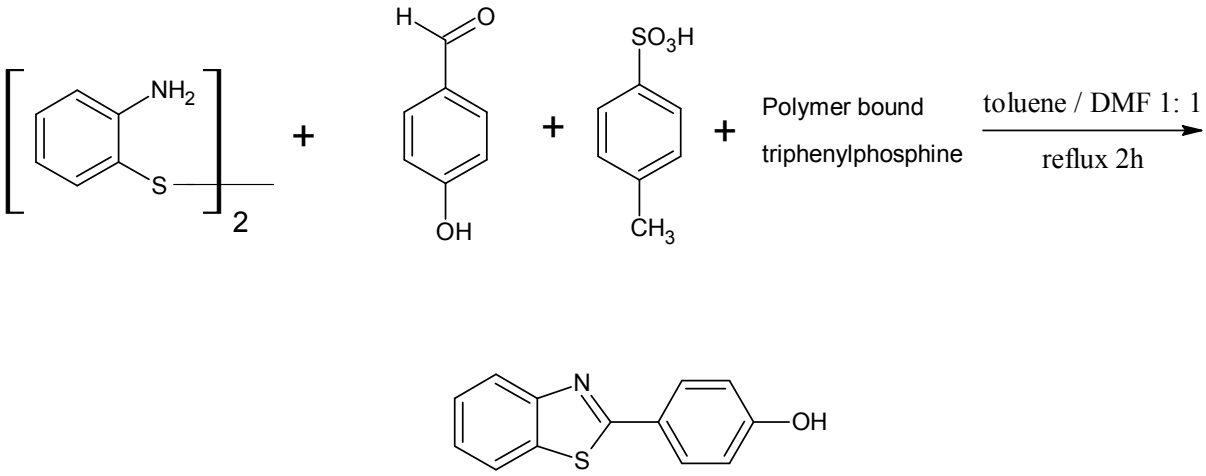


$C_{13}H_9NOS$

Mol. Wt.: 227.281

M.p.- 228-230°C

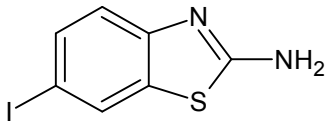
Prepared according to procedure A from 4-hydroxybenzaldehyde (0.183 g), polymer-bound triphenylphosphine (0.5 g), *p*-toluensulfonic acid(0,055 g) and and 2-aminothiophenol disulfide (0.38 g) in mixture of toluene and DMF 1:1 (5ml) as white solid in 58% yield.



^{1}H NMR (d_6-DMSO) d□10.30 (1H, s, OH), 8.07 (1H, d, *J* = 7.0 Hz, H-4), 7.98 (1H, d, *J* = 8.0 Hz, H-7), 7.93 (2H, d, *J* = 8.5 Hz, H-2', H-6'), 7.50 (1H, t, *J* = 7.5 Hz, H-5), 7.40 (1H, t, *J* = 7.5Hz, H-6), 6.94 (2H, d, *J* = 8.5 Hz, H-3', H-5').

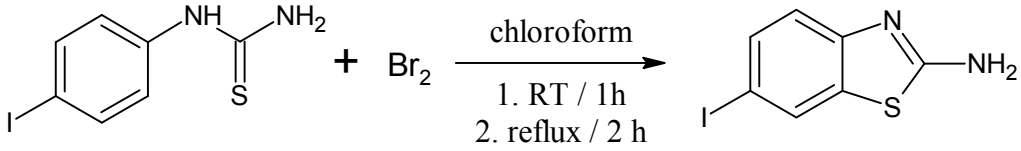
^{13}C-NMR (126MHz, $CDCl_3$) δ (ppm) 167.43, 160.49, 153.70, 134.08, 129.01, 126.38, 124.86, 124.03, 122.26, 122.06, 116.06

6-iodo-2-aminobenzothiazole- 112



$C_7H_5IN_2S$

Mol. Wt.: 276.09

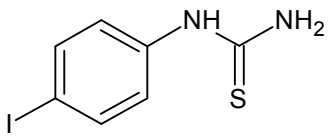


Prepared according to procedure G from 1-(4-iodophenyl)thiourea, 117, (0.5g ,) , solution of bromine(0.1ml , 1.8mmol) in chloroform(1ml) and chloroform(8ml) as white solid in 30% yield.

^{1}H NMR (500 MHz, DMSO) δ 8.01 (d, *J* = 1.8 Hz, 1H,H-7), 7.58 (s, 2H, NH_2), 7.49 (dd, *J* = 8.4, 1.8 Hz, 1H, H-5), 7.14 (d, *J* = 8.4 Hz, 1H, H-4).

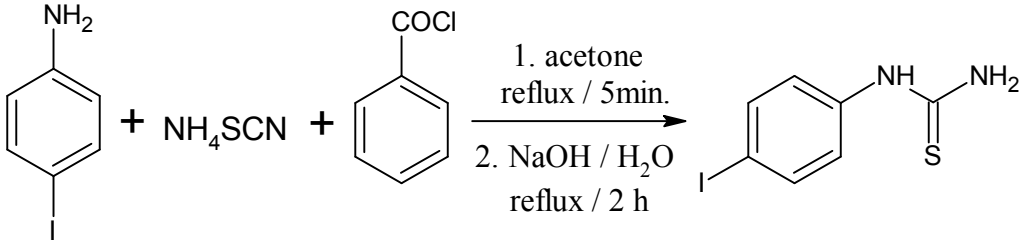
^{13}C NMR (126 MHz, DMSO) δ 166.97 , 152.44 , 133.94 , 133.53 , 128.79 , 119.65 , 82.93

1-(4-iodophenyl)thiourea- 117



$C_7H_5IN_2S$

Mol. Wt.: 278.11

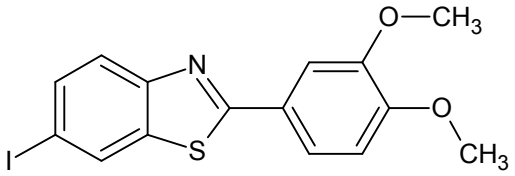


Prepared according to general procedure F from benzoilchloride (1.05g), ammonium thiocyanate (0.6g) and 4-iodoaniline (1.5g) in acetone (2ml) as white solid in 80% of yield.

^{1}H NMR (500 MHz, DMSO) δ 9.73 (s, 1H, NH), 7.67 – 7.63 (m, 2H, H-3,H-5), 7.29 (d, *J* = 8.7 Hz, 2H, H-2, H-6).

^{13}C NMR (126 MHz, DMSO) δ 181.07 , 139.15, 137.18, 124.97 , 88.59

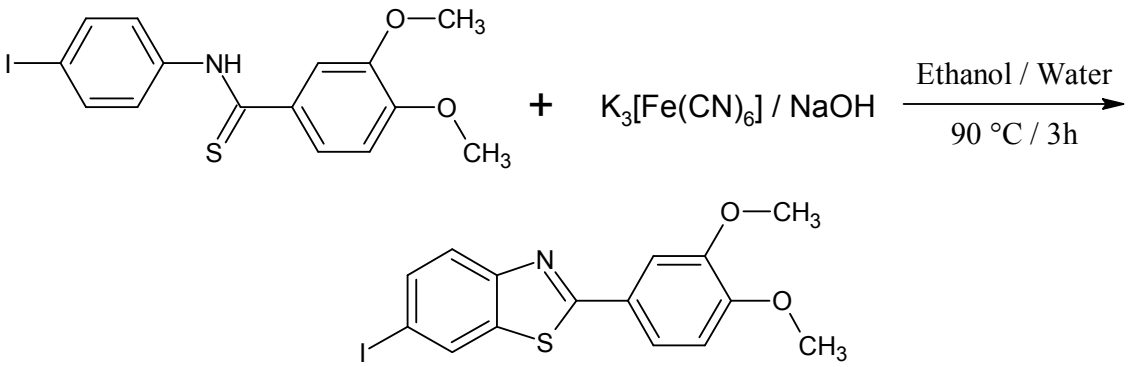
6-iodo-2-(3,4-dimethoxyphenyl)-1,3-benzothiazole- 121



$C_{15}H_{12}INO_2S$

Mol. Wt.: 397.230

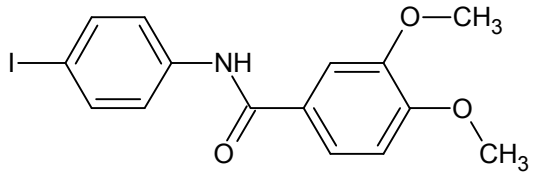
Prepared according to procedure D from *N*-4-iodophenyl-3,4-dimethoxy-thiobenzamide(1.3 g), 96% ethanol (2 ml), 30% water solution of NaOH (3.4 ml) andsolution of ferricyanide (4.29 g) in water(25 ml) as white solid in 45% of yield .



^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 (s, 1H, H-7), 7.76 (d, *J* = 0.8 Hz, 2H, H-2′,H-6′), 7.70 (d, *J* = 2.0 Hz, 1H, H-4), 7.58 (dd, *J* = 8.3, 2.1 Hz, 1H, H-5), 6.95 (d, *J* = 8.4 Hz, 1HH-5′), 4.03 (s, 3H, CH_3), 3.97 (s, 3H, CH_3).

^{13}C NMR (126 MHz, $CDCl_3$) δ 168.36, 153.55, 151.90, 149.42, 137.00, 135.33, 129.95, 126.12, 124.27, 121.28, 111.07, 109.82, 88.93, 56.15, 56.08.

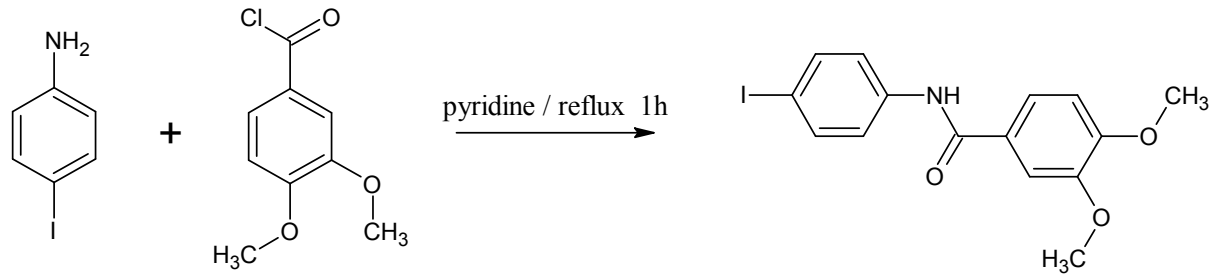
N-4-iodophenyl-3,4-dimethoxybenzamide- 124



$C_{15}H_{14}INO_3$

Mol. Wt.: 383.181

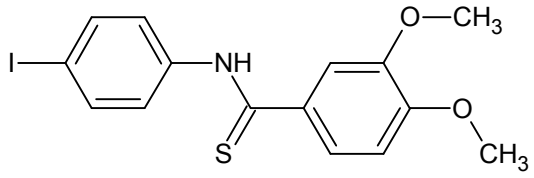
Prepared according to procedure B from the solution of 4,4-dimethoxybenzoylchloride(1 g) in pyridine(1ml) and solution of 4-iodoaniline (1.1 g) in pyridine(1.5 ml) as white fine crystals in 82% of yield.



^{1}H NMR (500 MHz, DMSO) δ 10.14 (s, 1H, CONH), 7.69 (d, *J* = 8.8 Hz, 2H, Ar), 7.64 – 7.60 (m, 3H, Ar, H-6), 7.53 (d, *J* = 2.0 Hz, 1H, H-2), 7.09 (d, *J* = 8.5 Hz, 1H, H-5), 3.85 (d, *J* = 1.5 Hz, 6H, OCH_3).

^{13}C NMR (126 MHz, DMSO) δ 165.12, 151.76, 148.42, 139.15, 137.19, 126.72, 122.55, 121.11, 111.08, 110.93, 87.04, 55.68

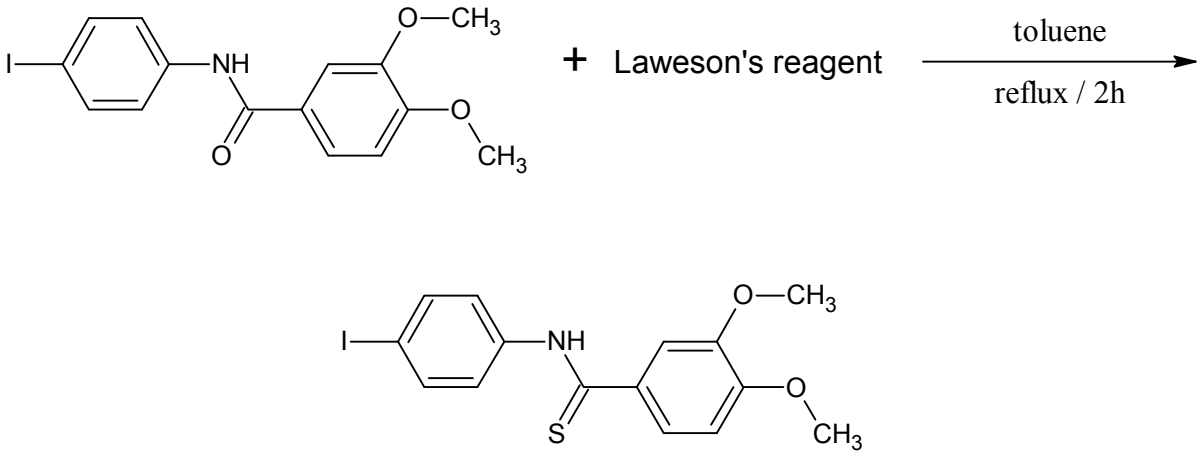
N-4-iodophenyl-3,4-dimethoxy-thiobenzamide- 126



$C_{15}H_{14}INO_2S$

Mol. Wt.: 399.246

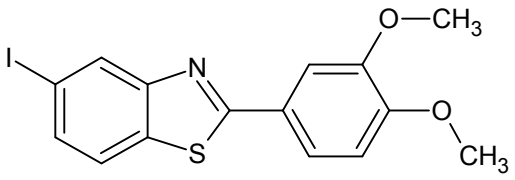
Prepared according to procedure C from N-4-iodophenyl-3,4-dimethoxy-benzamide,124,(1.54 g) and Lawesson´s reagent (0.8 g) in toluene(11 ml) as yellow crystals in 85% of yield.



^{1}H NMR (500 MHz, DMSO) δ 10.15 (s, 1H, CSNH), 7.70 (d, *J* = 8.8 Hz, 2H, Ar), 7.65 – 7.60 (m, 2H,H-5,H-6), 7.54 (d, *J* = 1.9 Hz, 2H, Ar), 7.09 (d, *J* = 8.5 Hz, 1H, H-2), 3.85 (d, *J* = 3.1 Hz, 6H, OMe).

^{13}C NMR (126 MHz, DMSO) δ 164.96, 151.75, 148.32, 139.16, 137.17, 126.67, 122.55, 121.00, 111.07, 110.91, 87.00, 55.66

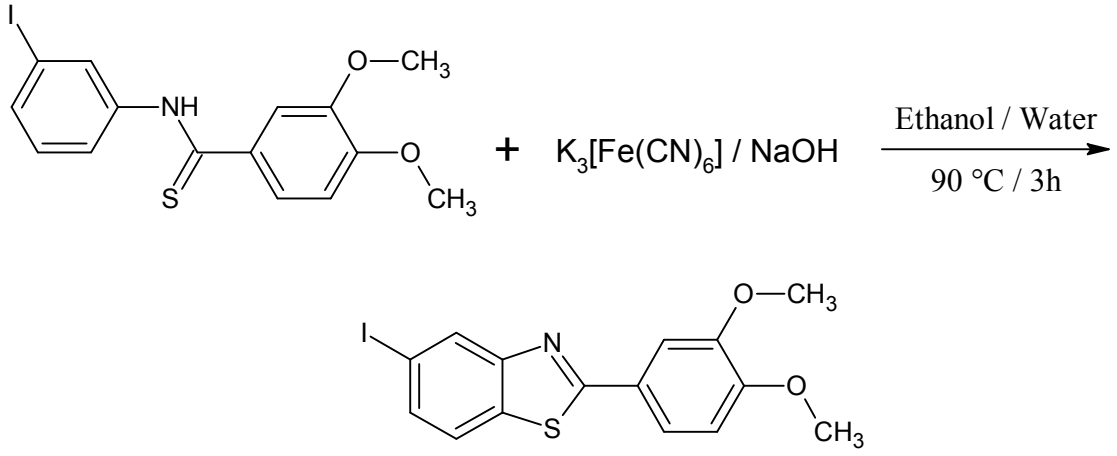
5-iodo-2-(3,4-dimethoxyphenyl)-1,3-benzothiazole- 128



$C_{15}H_{12}INO_2S$

Mol. Wt.: 397.230

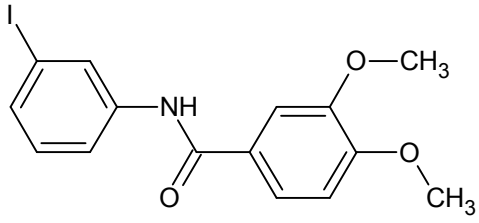
Prepared according to procedure D from *N*-3-iodophenyl-3,4-dimethoxy-thiobenzamide(1.13 g), 96% ethanol (1.5 ml), 30% water solution of NaOH (1.77 ml) and solution of potassium ferricyanide (3.7 g) in water(21 ml) as white solid in 66% of yield.



^{1}H NMR (500 MHz, $CDCl_3$) δ 8.40 (s, 1H, H-4), 7.99 (d, *J* = 8.1 Hz, 1H, H-6), 7.71 – 7.66 (m, 5H), 7.65 – 7.60 (m, 1H, H-2´), 7.22 (t, *J* = 7.9 Hz, 1H, H-6´), 6.97 (dd, *J* = 8.4, 4.3 Hz, 1H, H-5´), 4.04 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3).

^{13}C NMR (126 MHz, $CDCl_3$) δ 166.70, 151.91, 149.41, 142.90, 134.04, 127.59, 126.39, 122.36, 121.21, 111.10, 109.68, 83.69, 56.15, 56.08.

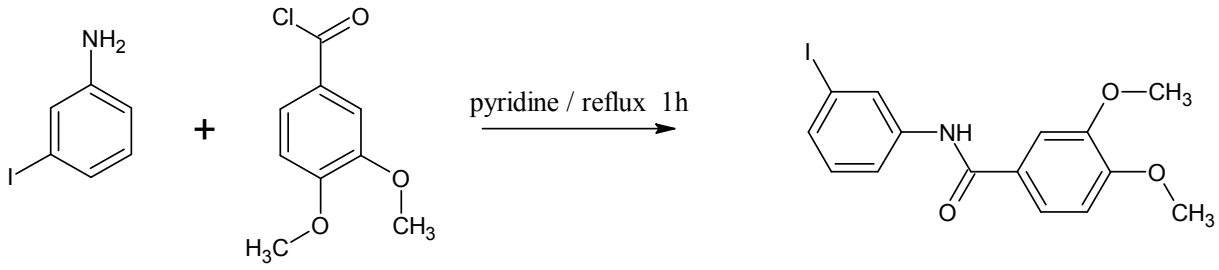
N-3-iodophenyl-3,4-dimethoxy-benzamide- 131



$C_{15}H_{14}INO_3$

Mol. Wt.: 383.181

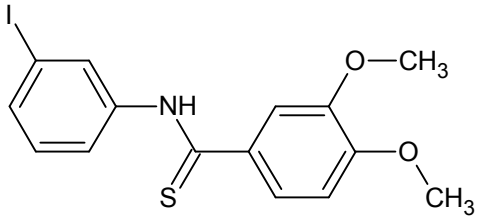
Prepared according to procedure B from the solution of 4,4-dimethoxybenzoylchloride(1 g) in pyridine(1ml) and solution of 4-iodoaniline (1.1 g) in pyridine(1.5 ml) as colourless oil in 57% of yield.



^{1}H NMR (500 MHz, DMSO) δ 10.12 (s, 1H, CONH), 7.81 (dt, *J* = 7.6, 3.8 Hz, 1H, Ar), 7.63 (dd, *J* = 8.4, 2.1 Hz, 1H, Ar), 7.54 (d, *J* = 2.0 Hz, 1H), 7.45 (dd, *J* = 7.8, 0.5 Hz, 1H), 7.16 (t, *J* = 8.0 Hz, 1H), 7.10 (d, *J* = 8.5 Hz, 1H), 3.85 (d, *J* = 3.5 Hz, 6H).

^{13}C NMR (126 MHz, DMSO) δ 165.12, 151.76, 148.42, 139.15, 137.19, 126.72, 122.55, 121.11, 111.08, 110.93, 87.04, 55.68

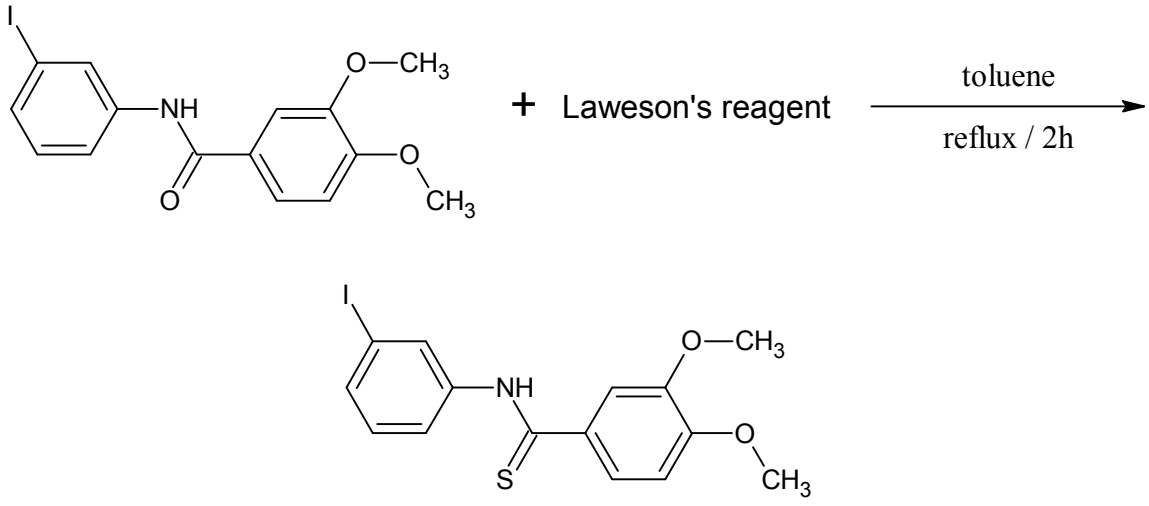
N-(3-iodophenyl)-3,4-dimethoxybenzenecarbothioamide- 132



$C_{15}H_{14}INO_2S$

Mol. Wt.: 399.246

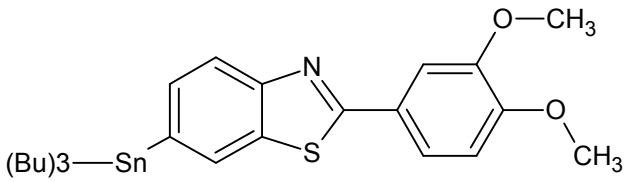
Prepared according to procedure C from *N*-3-iodophenyl-3,4-dimethoxy-benzamide(1.9 g) and Lawesson´s reagent (1.1 g) in toluene(14 ml) as orange crystals in 57% of yield.



^{1}H NMR (500 MHz, DMSO) δ 11.51 (s, 1H), 8.21 (s, 1H), 7.82 (d, *J* = 6.8 Hz, 1H), 7.64 (d, *J* = 7.9 Hz, 1H), 7.55 (d, *J* = 8.2 Hz, 2H), 7.24 (t, *J* = 8.0 Hz, 1H), 7.05 (d, *J* = 8.1 Hz, 1H), 3.85 (s, 6H).

^{13}C NMR (126 MHz, DMSO) δ 196.88, 151.58, 147.76, 141.48, 134.57, 134.30, 132.74, 130.36, 124.00, 121.01, 111.44, 110.63, 93.64, 55.67.

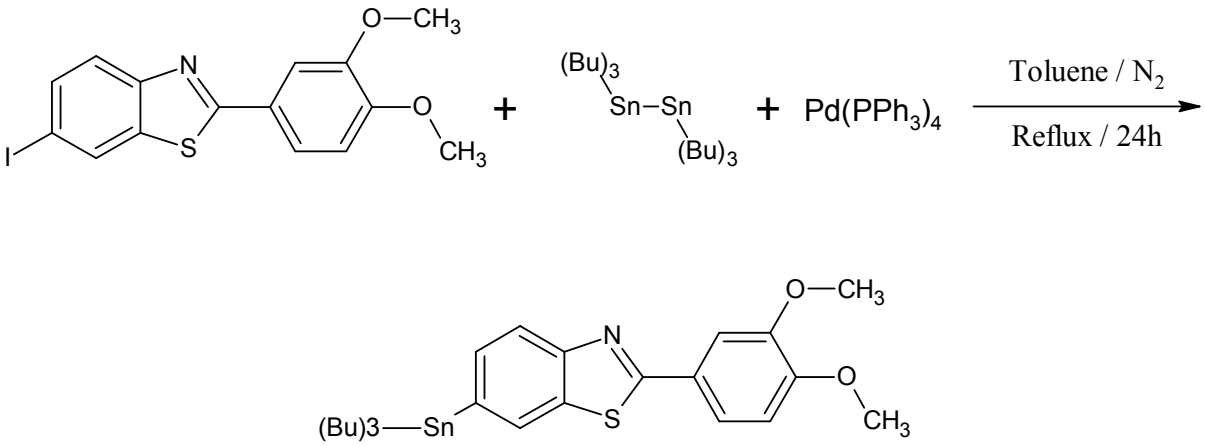
6-(tributylstannyl)-2-(3,4-dimethoxyphenyl)-1,3-benzothiazole- 133



$C_{27}H_{39}NO_2SSn$

Mol. Wt.: 560.379

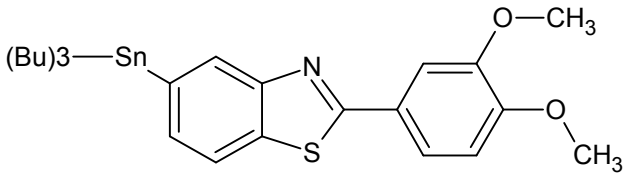
Prepared according to procedure E from 6-iodo-2-(3,4-dimethoxyphenyl)-1,3-benzothiazole (0.5 g), bis(tributyltin) (1.27 ml)and tetrakis(triphenylphosphine)palladium (0,01 g) in anhydrous toluene(8 ml), purified by column chromatography (silica gel / Hexane: Ethylacetate – 9:1) affording product as colouress oil in 53% of yield.



^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04 – 8.01 (m, 1H, H-4), 7.99 (s, 1H, H-7), 7.75 (d, *J* = 1.9 Hz, 1H, H-5), 7.63 (dd, *J* = 8.3, 2.0 Hz, 1H, H-6′), 7.57 (d, *J* = 8.0 Hz, 1H, H-5′), 6.96 (d, *J* = 8.4 Hz, 1H, H-2′), 4.05 (s, 3H, OMe), 3.97 (s, 3H, OMe), 1.65 – 1.57 (m, 6H, CH_2), 1.43 – 1.33 (m, 6H, CH_2), 1.18 – 1.13 (m,6H, CH_2), 0.96 – 0.90 (m, 9H, CH_3).

^{13}C NMR (126 MHz, $CDCl_3$) δ 167.35, 154.07, 151.54, 149.36, 138.68, 135.14, 133.71, 128.96, 126.90, 122.26, 121.13, 111.05, 109.90, 56.14, 56.04, 29.10, 27.37, 13.67, 9.87.

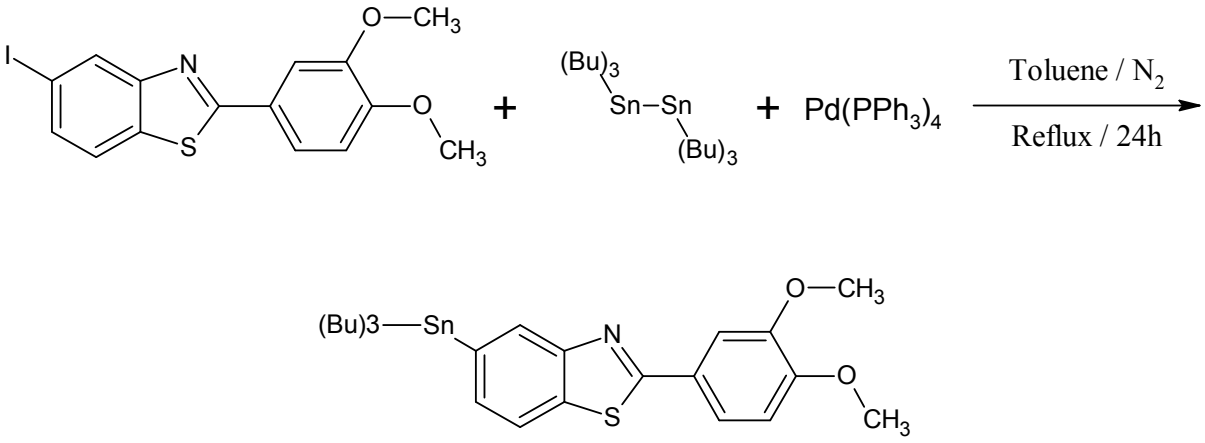
5-(tributylstannyl)-2-(3,4-dimethoxyphenyl)-1,3-benzothiazole- 134



$C_{27}H_{39}NO_2SSn$

Mol. Wt.: 560.379

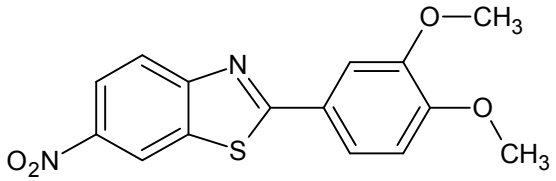
Prepared according to procedure E from N-3-iodophenyl-3,4-dimethoxy-thiobenzamide cyclisation (0,75 g), bis(tributyltin) (1,92 ml) and tetrakis(triphenylphosphine)palladium (0,015 g) in anhydrous toluene(12 ml) purified by column chromatography (silica gel / Hexane : Ethylacetate – 9:1) affording product as colourless oil in 30% of yield.



^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99 (dd, *J* = 7.5, 1.8 Hz, 1H), 7.75 (d, *J* = 2.0 Hz, 1H), 7.64 (dd, *J* = 8.3, 2.0 Hz, 1H), 7.47 – 7.42 (m, 2H), 6.98 (d, *J* = 8.4 Hz, 1H), 4.06 (s, 3H), 3.98 (s, 3H), 1.62 (ddd, *J* = 12.6, 9.5, 6.5 Hz, 6H), 1.43 – 1.35 (m, 6H), 1.29 – 1.24 (m, 6H), 0.92 (t, *J* = 7.3 Hz, 9H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 167.16, 152.60, 151.47, 149.37, 144.17, 134.63, 133.51, 127.03, 126.12, 122.63, 121.15, 111.04, 109.82, 56.17, 56.05, 29.28, 27.33, 13.64, 9.96

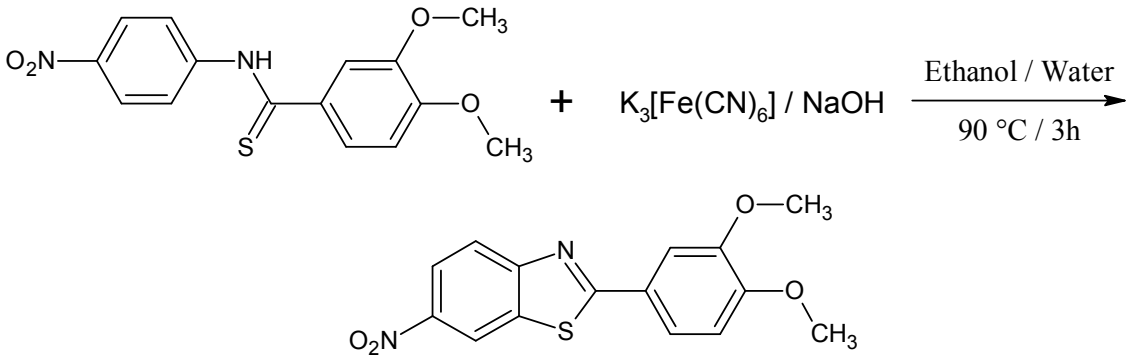
6-nitro-2-(3,4-dimethoxyphenyl)-1,3-benzothiazole- 141



$C_{15}H_{12}N_2O_4S$

Mol. Wt.: 316.331

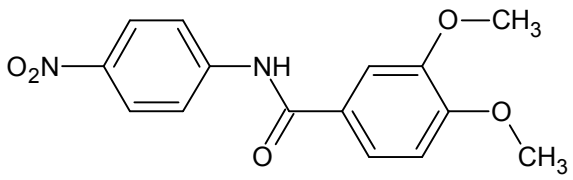
Prepared according to procedure D from N-4-nitrophenyl-3,4-dimethoxy-thiobenzamide(3.3 g), 96% ethanol(2 ml), 10% water solution of NaOH (15 ml) and solution of potassium ferricyanide (13.6 g) in water(26 ml) as yellow solid in 62% of yield .



^{1}H NMR (500 MHz, $CDCl_3$) δ 7,95 (d, J=8Hz, 1H, H-4), 7.75(s, 1H, H-7), 7,67(s, 1H,H-2′), 7,59 (d, J=7 Hz,1H, H-5), 7,30 (d, J=8,5Hz, 1H, H-6′), 6,95 (d, J=8,5Hz, 1H, H-5′), 4,03(s, 3H, OMe), 3,96(s, 3H, OMe)

^{13}C NMR (126 MHz, $CDCl_3$) δ 166.07, 151.72, 151.44, 149.10, 134.83, 134.83, 134.42, 127.91, 125.72, 122.02, 121.66, 120.65, 111.91, 109.35, 55.69, 20.98

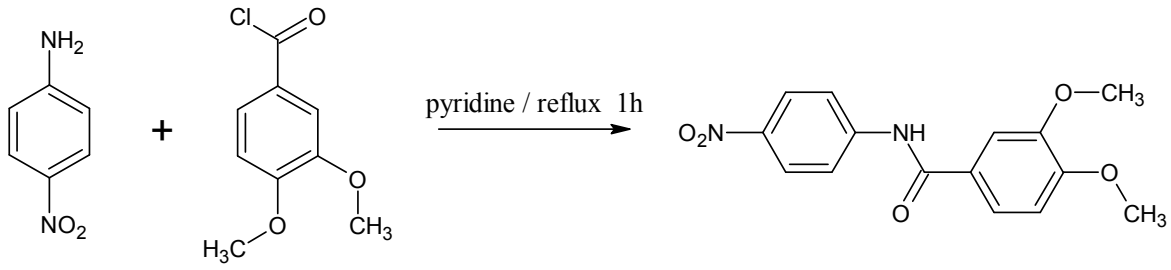
3,4-dimethoxy-*N*-(4-nitrophenyl)benzamide- 145



$C_{15}H_{14}N_2O_5$

Mol. Wt.: 302.28

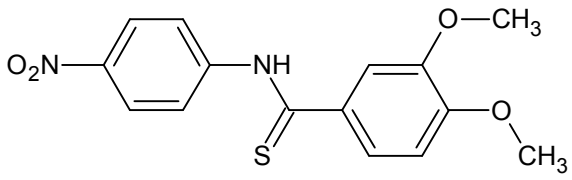
Prepared according to procedure B from the solution of 4,4-dimethoxybenzoylchloride(1 g) in pyridine(1ml) and solution of 4-nitrooaniline (0.69 g) in pyridine(1,5 ml) as brown solid in 71% of yield.



^{1}H NMR (500 MHz, DMSO) δ 10.61 (s, 1H, CONH), 8.28 (d, *J* = 9.3 Hz, 2H, Ar), 8.06 (dd, *J* = 9.8, 2.5 Hz, 2H, Ar), 7.67 (dd, *J* = 8.4, 2.1 Hz, 1H, H-6), 7.56 (d, *J* = 2.0 Hz, 1H, H-2), 7.13 (d, *J* = 8.5 Hz, 1H, H-5), 3.87 (d, *J* = 0.4 Hz, 6H, OMe).

^{13}C NMR (126 MHz, DMSO) δ 165.54, 152.17, 148.38, 145.67, 142.26, 126.14, 124.70, 121.51, 119.76, 111.22, 110.93, 55.71, 55.65.

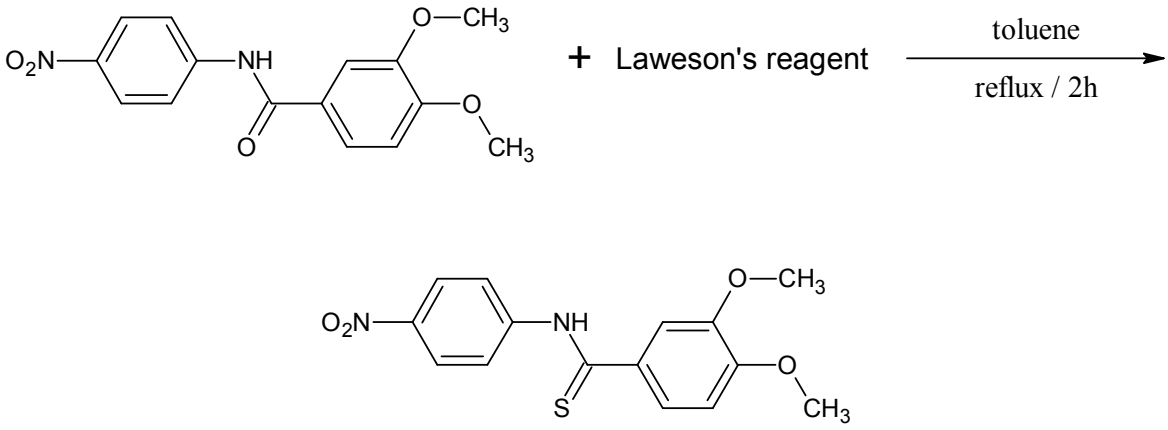
N-4-nitrophenyl-3,4-dimethoxy-thiobenzamide- 146



$C_{15}H_{14}N_2O_4S$

Mol. Wt.: 318.347

Prepared according to procedure C from N-4-nitrophenyl-3,4-dimethoxy-benzamide (4.2 g) and Lawesson´s reagent (2.81 g) in toluene(28 ml) as brown crystals in 77% of yield.



^{1}H NMR (500 MHz, DMSO) δ 11.87 (s, 1H), 8.30 (d, *J* = 9.0 Hz, 2H), 8.19 (d, *J* = 8.9 Hz, 2H), 7.55 (d, *J* = 8.0 Hz, 2H), 7.07 (d, *J* = 8.2 Hz, 1H), 3.86 (d, *J* = 3.1 Hz, 6H).

^{13}C NMR (126 MHz, DMSO) δ 197.91, 151.90, 147.84, 146.09, 143.97, 134.62, 124.13, 124.01, 121.14, 111.61, 110.64, 55.78, 55.60.

6. References

1 Naiki,H.; Higuchi, K.; Hosokawa,M.; Takeda, T.
Fluorometric determination of amyloid fibrils *in vitro* using the flurescent dye, Thioflavin T.
Anal.Biochem.,**1989**,*177*, 244–249

2 Khurana, R.; Coleman, C.;Ionescu-Zanetti,C.; Carter, S. A.; Krishna,V.; Grover,R. K.; Roy, R.; Singh, S.
Mechanism of thioflavin T binding to amyloid fibrils.
J. Struct. Biol., **2005**,*151*, 229–238

3 Song, J. H.; Huang , C. S.; Nagata, K.;Yeh, J. Z.;Narahashi, T.;
Differential action of riluzole on tetrodotoxin-sensitive and tetrodotoxin-resistant sodium channels,
J. Pharmacol..Exp.Ther. **1997**, *282*, 707–14.

4 Rilutek 50 mg film-coated tablets,SPC .http://www.medicines.org.uk/emc/medicine/1672/spc
accessed 10.5, **2013**

5 http://www.alzheimers.org.uk: accessed 12.3, **2013**

6 http://www.uzis.cz/en/fast-information/health-care-patients-treated-dementia-out-patient-and-patient-facilities-czech-republic-2007-2011 : accessed 12.3, **2013**

7 Svedberg,M. M.; Rahman,O.; Hall,H.
Preclinical studies of potential amyloid binding PET/SPECT ligands in Alzheimer's disease.
Nucl. Med. Biol., **2012** ,*39*, 484-501

8 Mathis, C. A.; Wang, Y.; Holt, D. P.; Huang, G-F.; Debnath, M. L.; Klunk, W. E.
Synthesis and evaluation of ^{11}C-labeled 6-substituted 2-arylbenzothiazoles as amyloid imaging agents.
*J. Med. Chem.***2003**, *46*, 2740-2754.

9 Klunk, W.E.; Engler, H.; Nordberg, A.; Wang, Y.; Blomqvist, G.; Holt, D.P.; Bergström, M.; Savitcheva, I.; Huang, G-F.; Estrada, S.; Ausén, B.; Debnath, M.L.; Barletta, J.; Price, J.C.; Sandell, J.; Lopresti, B.J.; Wall, A.; Koivisto, P.; Antoni, G.; Mathis, C.A.; Långström, B.
Imaging brain amyloid in alzheimer's disease with pittsburgh compound-B.
Ann. Neurol. **2004**, *55*, 306-319.

10 Klunk WE, Mathis CA. Whatever happened to Pittsburgh compound A?
Alzheimer Dis. Assoc. Disord. **2008**, *22*, 198–203.

11 Serdons, K.;Verduyckt,T.;Vanderghinste, D.;Borghgraef, P.;Cleynhens,J.; Van Leuven, F.; Kung, H.;Bormans, G.; Verbruggen, A.
^{11}C-labelled PIB analogues as potential tracer agents for *in vivo* imaging of amyloid β in Alzheimer's disease
*Eur. J. Med. Chem.,***2009**, *44*,1415–1426

12 Nelissen, N.; Van Laere, K.;Thurfjell, L.;Owenius, R.;Vandenbulcke M.;Koole, M.; *et al.*
Phase 1 study of the Pittsburgh compound Bderivative [^{18}F]-flutemetamol in healthy volunteers and patients withprobable Alzheimer disease.
J. Nucl. Med. **2009**, *50*, 1251–1259

13 Hall, H.; Svedberg, M.; Estrada, S.;Rahman, O.;Ulin, J.; Nilsson, L.; *et al.*
Amyloid binding ligand properties and Abeta peptide levels inAlzheimer's disease, studies in postmortem brain tissue and intransgenic mice.
Abstract at International Conference on Alzheimer'sdisease, Honolulu, HI, USA; **2010**

14 Serdons, K.; Van Laere, K.; Janssen, P.; Kung, H. F.;Bormans, G.; Verbruggen, A.;
Synthesis and evaluation of three [^{18}F]-labeledaminophenylbenzothiazoles as amyloid imaging agents.
*J. Med. Chem.***2009**, 52, 7090–7102.

15 Berndt, U.; Stanetty, C.; Wanek, T.; Kuntner, C.; Stanek, J.; Berger, M.; Bauer, M.; Henriksen, G.; Wester, H-J. Kvaternik, H. Angelberger, P.; Noe, C.
Synthesis of a [^{18}F]-fluorobenzothiazole as potential amyloid imaging agent.
J. Labelled Compd. Radiopharm.. **2008**, *51*, 137-145.

16 Neumaier,B.;Deisenhofer, S.;Sommer,C.; Solbach,C.; S.N. Reske, S. N.;Mottaghy, F.
Synthesis and evaluation of [^{18}F]-fluoroethylated benzothiazole derivatives for *in vivo* imaging of amyloid plaques in Alzheimer's disease
Appl. Radiat. Isot. **2010**,*68*, 1066–1072

17 Chen,X.; Yu,P.; Zhang,L.; LiuB.
Synthesis and biological evaluation of 99mTc, Re-monoamine-monoamide conjugated to 2-(4-aminophenyl)benzothiazole as potential probes for β-amyloid plaques in the brain
Bioorg. Med. Chem. Lett. **2008**, *18*, 1442–1445

18 Pan, J.; Mason, N.S.;Debnath, M.L.; Mathis, C. A.;Klunk, W. E.; Lin, K-S.;
Design,synthesis and structure-activity relationship of rhenium 2-arylbenzothiazoles as β-amyloid plaque binding agents,
Bioorg. Med. Chem. Lett. **2013**, -in press

19 Zhuang, Z-P.; Kung, M-P.; Hou, C.; Skovronsky, D.M.; Gur, T.L.; Plőssl, K.; Trojanowski, J.Q.; Lee, V.M-Y.; Kung, H.F.
Radioiodinatedstyrylbenzenes and thioflavins as probes for amyloid aggregates.
J. Med. Chem. **2001**, *44*, 1905-1914.

20 Kung, M. P.;Skovronsky, D. M.;Hou, C.;Zhuang, Z.P.;Gur, T. L.; Zhang , B.;Trojanowski, J.Q.; Lee, V. M.; Kung, H. F.
Detection of amyloid plaques by radioligands for Abeta40 and Abeta42: potential imaging agents in Alzheimer's patients.
J. Mol. Neurosci.**2003**, *20*, 15-24.

21 Cui, M.C.; Li, Z.J.; Tang, R.K.; Liu, B.L.
Synthesis and evaluation of novel benzothiazole derivatives based on the bithiophene structure as potential radiotracers for β-amyloid plaques in Alzheimer's disease
Bioorg. Med. Chem. **2010**,*18*, 2777–2784

22 http://www.cancerresearchuk.org , accesed 28.3.**2013**

23 http://www.uzis.cz/category/tematicke-rady/zdravotnicka-statistika/novotvary , accesed 28.3.**2013**

24 Shi, D-F.; Bradshaw, T.D.; Wrigley, S.; McCall, C.J. ;Lelieveled, P.; Fichtner, I.; Stevens, M.F.G. Antitumor benzothiazoles. 3. synthesis of 2-(4-aminophenyl) benzothiazoles and evaluation of their activities against breast cancer cell lines *in vitro* and *in vivo*.
J. Med. Chem. **1996,** *39,* 3375-3384.

25 Bradshaw, T.D.; Wrigley, S.; Shi, D-F.; Schultz, R.J.;Paull, K.D.; Stevens, M.F.G.
2-(4-Aminophenyl)benzothiazoles: novel agents with selective profiles of *in vitro* and *in vivo* activity
*Brit. J. Cancer***1998**, *77*,745.

26 Bradshaw, T.D.; Shi, D-F.; Schultz, R.J.; Paull, K.D.;Kelland, L.; Wilson, A.; Garner, C.; Fiebig, H.H.;Wrigley, S.; Stevens, M.F.G.
Influence of 2-(4-aminophenyl)benzothiazoles on growth of human ovarian carcinoma cell lines *in vitro* and *in vivo*.
*Brit. J. Cancer***1998**, *78*,421-429.

27 Bradshaw, T.D.; Stevens, M.F.G.; Westwell, A.D.
The Discovery of the Potent and Selective Antitumour Agent 2-(4-Amino-3-ethylphenyl)benzothiazole (DF 203) and Related Compounds
*Curr.Med.Chem.***2001**, *8*, 203-210.

28 Kashiyama, E.; Hutchinson, I.; Chua, M-S.; Stinson, S.F.;Phillips,L.R.;Kaur, G.;Sausville, E.A.; Bradshaw, T.D.; Westwell, A.D.; Stevens, M.F.G.
Antitumor benzothiazoles. 8. Synthesis, metabolic formation and biological properties of the C-and N-oxidation products of antitumor 2-(4-aminopheny)benzothiazoles.
*J. Med. Chem.***1999**, *42*, 4172-4184.

29 Chua, M-S.; Kashiyama, E.; Bradshaw, T.D.; Stinson, S.F.;Brantley, E.; Sausville, E.A.; Stevens,M.F.G.
Role of CYP1A1 in modulation of antitumor properties of the novel agent 2-(4-amino-3-methylphenyl)benzothiazole (DF 203, NSC 674495) in human breast cancer cells.
*Cancer Res.***2000**,*60*, 5196-5203.

30 Hose, C.; Riviera, M.; Sausville, E.A.; Monks, A.
Induction of cytochrome P450 CYP1A1 and cytochrome P450 CYP1B1 by 2-(4-amino-3-methylphenyl)benzothiazole (BZ) in 60 human tumour cell lines: correlation with BZ toxicity.
*Proc. Am. Assoc.Cancer Res.***2001**, *42*,511.

31 Hutchinson, I.; Chua, M.-S.; Browne, H. L.; Trapani, V.; Bradshaw,T. D.; Westwell, A. D.; Stevens, M. F. G.
Antitumor benzothiazoles14. Synthesis and *in vitro* biological properties of fluorinated 2-(4-aminophenyl)benzothiazoles.
J. Med. Chem. **2001**, *44*, 1446-1455

32 Bradshaw, T.D.; Bibby, M.C.; Double, J.A.; Fichtner, I.; Cooper,P.A.; Alley, M.C.; Donohue, S.; Stinson, S.F.; Tomaszewjski, J.E.;Sausville, E.A.; Stevens, M.F.G
Preclinical evaluation of amino acid prodrugs of novel antitumor 2-(4-amino-3-methylphenyl)benzothiazoles.
*Mol. Cancer Therap.***2002**, *1*,239-246

33 Bradshaw, T.D.; Westwell, A.D.
The development of the antitumourbenzothiazole prodrug, phortress, as a clinical candidate.
Curr. Med. Chem. **2004,***11*, 1009-1021.

34 Wells,G.; Bradshaw, T. D.; Diana,P.; Seaton, A.; Shi,D.-F.; Westwell,A. D.; Stevens, M.F.G.
Antitumourbenzothiazoles. Part 10: The synthesis and antitumour activity of benzothiazole substituted quinol derivatives
Bioorg. Med. Chem. Lett. **2000**, 10, 513–515

35 Hutchinson, I.; Bradshaw,T. D.; Matthews,C. S.; Stevens,M F.G.; Westwell, A. D.
Antitumourbenzothiazoles. Part 20:† 3′-Cyano and 3′-Alkynyl-Substituted 2-(4′-Aminophenyl)benzothiazoles as new potent and selective analogues
Bioorg. Med. Chem. Lett. **2003**,*13*, 471–474

36 Mortimer, C.G.; Wells, G.; Crochard, J-P.; Stone, E.L.; Bradshaw, T.D.; Stevens, M.F.G.; Westwell, A.D.
Antitumor benzothiazoles. 26. [1]2-(3,4-dimethoxyphenyl)-5-fluorobenzothiazole (GW 610, NSC 721648), a simple fluorinated 2-arylbenzothiazole, shows potent and selective inhibitory activity against lung, colon, and breast cancer cell lines.
*J. Med. Chem.***2006**, *49*, 179-185.

37 Walker, J.; Nitiss, J. L.
DNA Topoisomerase II as a Target for Cancer Chemotherapy
Cancer Invest. **2002**, *20*, 570-589.

38 Choi, S.J.; Park,H. J.; Lee,S. K.; Kim,S.W.; Han,G.; Choo, H.Y.
Solid phase combinatorial synthesis of benzothiazoles and evaluation of topoisomerase II inhibitory activity
Bioorg. Med. Chem. **2006**, *14*, 1229–1235

39 Henriksen, G.; Hauser, A.I.; Westwell, A.D.; Yousefi, B.H.; Schwaiger, M.; Drzezga, A.; Wester, H-J. Metabolically stabilized benzothiazoles for imaging of amyloid plaques.
*J. Med. Chem.***2007**, *50*, 1087-1089.

40 Wang, Y.; Mathis, C.A.; Huang, G-F.; Debnath, M.L.; Holt, D.P.; Shao, L.; Klunk, W.E.
Effects of lipophilicity on the affinity and non-specific binding of iodinated benzothiazole derivatives.
J. Mol. Neurosci. **2003**, *20*, 255-260

41 Harrop, T.C.; Rodriguez, K.; Mascharak, P.K.
Convenient one-pot synthesis of N,N'-bis(2-mercaptophenyl)pyridine-2,6-dicarboxamide and N-2-mercaptophenyl-2'-pyridinecarboxamide without protection of the thiol group(s).
*Synth. Comm.***2003**, *33*, 1943-1949.

42 Chakraborti, A.K.; Rudrawar, S.; Kaur, G.; Sharma, L. An efficient conversion of phenolic esters to benzothiazoles under mild and virtually neutral conditions.
Synlett **2004**,*9*, 1533-1536

43 Wu, C.; Cai, L.; Wei, J.; Pike, V.W.; Wang, Y. Lipophilic analogs of thioflavin S as novel amyloid-imaging agents.
Curr. Alzheimer Res. **2006**, *3*, 259-266.

44 Taneda, M.; Kodama, Y.; Eda, Y.; Koyama, H.; Toshio, K.
Photoisomerization of 2-(2-hydroxyphenyl)benzothiazole derivatives as an adequate model to reveal photochromism of Schiff bases in the solid state.
Chem. Lett. **2007**, *36*, 1410-1411.

45 Suh, J.; Yoo, S.H.; Kim, M.G.; Jeong, K.; Ahn, J.Y.; Kim, M-S.; Chae, P.S.; Lee, T.Y.; Lee, J.; Lee, J.; Jang, J.A.; Ko, E.H.
Cleavage agents for soluble oligomers of amyloid peptides.
Ang. Chem. Int. Ed. **2007**, *46*, 7064-7067.

46 Charris, J.; Camacho, J.; Ferrer, R.; Lobo, G.; Barazarte, A.; Gamboa, N.; Rodrigues, J.; Lopez, S.
A convenient route to 2-substituted benzothiazole-6-carboxylic acids using nitrobenzene as oxidant.
J. Chem. Res. **2006**,*12*, 769-770.

47 Chang, W-C.; Hu, A.T.; Duan, J-P.; Rayabarapu, D.K.; Cheng, CH.
Colortunable phosphorescent light-emitting diodes based on iridium complexes with substituted 2-phenylbenzothiazoles as the cyclometalated ligands.
J. Organomet. Chem. **2004**, *689*, 4882-4888

48 Morakot, N.; Tomapatanaget, B.; Ngeon-Tae, W.; Aeungmaitrepirom, W.; Tuntulani, T.
Synthesis and fluorescence sensing properties of calix[4]arenes containing fluorophores.
*Supramol. Chem.***2005**, *17*, 655-659.

49 Racane, L.; Stojkovil, R.; Tralic-Kulenovic, V.; KarminskiZamola, G.
Synthesis and antitumor evaluation of novel derivatives of 6-amino-2-phenylbenzothiazoles.
*Molecules***2006**, *11*, 325-333.

50 Prutap, U.R.; Mali, J.R.; Jawale, D.V.; Mane, R.A.
Bakers' yeast catalyzed synthesis of benzothiazoles in an organic medium
*Tetrahedron Lett.***2009**,*50*, 1352–1354

51 Bandyopadhyay, P.; Sathe, M.; Prasad, G.K.; Sharma, P.; Kaushik, M.P.;
Mesoporous mixed metal oxide nanocrystals: Efficient and recyclable heterogeneous catalysts for the synthesis of 1,2-disubstituted benzimidazoles and 2-substituted benzothiazoles
J. Mol. Catal. A: Chem . **2011**, *341*,77–82

52 Aiello, S.; Wells, G.; Stone, E. L.; Kadri, H.; Bazzi, R.; Bell, D. R.; Stevens, M. F. G.;
Matthews, C. S.; Bradshaw, T. D.; Westwell, A. D.
Synthesis and Biological Properties of Benzothiazole, Benzoxazole, and Chromen-4-oneAnalogues of the Potent Antitumor Agent 2-(3,4-Dimethoxyphenyl)-5-fluorobenzothiazole (PMX 610, NSC 721648).
J. Med. Chem. **2008**, *51*,5135-5139.

53 Weekes, A. A. *Ph.D. Thesis*, Cardiff University, U.K., **2010**

54 Wang, M.; Gao, M,; Mock, B.H.; Miller, K.D.; Sledge, G.W.; Hutchins, G.D.; Zheng, Q.H.
Synthesis of carbon-11 labeled fluorinated 2-arylbenzothiazoles as novel potential PET cancer imaging agents
Bioorg. Med. Chem. **2006** ,*14*,8599 - 8607

55 Weekes,A. A.; Bagley,M. C.; Westwell, A.D.;
An efficient synthetic route to biologically relevant 2-phenylbenzothiazoles substituted on the benzothiazole ring
Tetrahedron **2011**,*67*, 7743–7747

56 Chakraborti, A.K.; Rudrawar, S.; Kaur, G.; Sharma, L.
An efficient conversion of phenolic esters to benzothiazoles under mild and virtually neutral conditions.
Synlett **2004**, *9*, 1533-1536.

57 Mohammadpoor-Baltork, I.; Khosropour,A. R.; Hojati, S.F.
$ZrOCl_2{\cdot}8H_2O$ as an efficient, environmentally friendly and reusable catalyst for synthesis of benzoxazoles, benzothiazoles, benzimidazoles and oxazolo[4,5-b]pyridines under solvent-free conditions.
Catal. Comm. **2007**, *8*, 1865-1870.

58 Bhuva ,H.A.; Kini, S.G.
Synthesis, anticancer activity and docking of some substituted benzothiazoles as tyrosine kinase inhibitors
J. Mol. Graphics Modell. **2010**, *29*, 32–37

59 Kamal, A.; Kumar, B.A.; Suresh, P.; Shankaraiah, N.; Kumar, M.S.
An efficient one-pot synthesis of benzothiazolo-4β-anilino-podophyllotoxin congeners: DNA topoisomerase-II inhibition and anticancer activity
Bioorg. Med. Chem. Lett. **2011**, *21*,350–353

60 Downer-Riley, N.K.; Jackson, Y.A.
Iodine-mediated cyclisation of thiobenzamides to produce benzothiazoles and benzoxazoles. *Tetrahedron* **2007**, *63*, 10276-10281.

61 Bose, D.S.; Idrees, M.
Hypervalent iodine mediated intramolecularcyclisation of thioformanilides: expeditious approach to 2-substitutedbenzothiazoles.
*J. Org. Chem.***2006**, *71*, 8261-8263.

62 Bose, D.S.; Idrees,M.; Todewale, I.K.; Jakka, N.M.; Rao, J.V.
Hybrids of privileged structures benzothiazoles and pyrrolo[2,1-c] [1,4]benzodiazepin-5-one, and diversity-oriented synthesis of benzothiazoles
Eur. J. Med. Chem. **2012**,*50*,27–38

63 Hutchinson,I.; Stevens,M.F.G.; Westwell, A. D.
The regiospecific synthesis of 5- and 7-monosubstituted and 5,6-disubstituted 2-arylbenzothiazoles
Tetrahedron Lett, **2000**,*41*, 425–428

64 Organ, M.G.; Abdel-Hadi, M.; Avola, S.; Hadei, N.; Nasielski, J.; O'Brien, C.J.; Valente, C.
Biaryls made easy: PEPPSI and the Kumada-Tamao-Corriu reaction.
Chem. Eur. J. **2007**, *13*, 150-157.

65 Rueping, M.; Ieawsuwan, W.
A manganese-catalyzed crosscoupling reaction.
*Synlett***2007,***2*,247-250.

66 Majo, V.J.; Prabhakaran, J.; Mann, J.J.; Kumar, J.S.D.
An efficient palladium catalyzed synthesis of 2-arylbenzothiazoles.
Tetrahedron Lett. **2003**, *44*, 8535-8537.

67 Liebeskind, L.S.; Srogl, J.
Heteroaromatic thioether-boronic acid cross-coupling under neutral reaction conditions.
Org. Lett. **2002**, *4*, 979-981.

68 Heo, Y.; Song, Y.S.; Kim, B.T.; Heo, J-N.
A highly regioselective synthesis of 2-aryl-6-chlorobenzothiazoles employing microwavepromoted Suzuki-Miyaura coupling reaction.
*Tetrahedron Lett.***2006**, *47*, 3091-3094.

69 Belfield, K.D.; Morales, A.R.; Kang, B-S.; Hales, J.M.; Hagan, D.J.; van Stryland, E.W.; Chapela, V.M.; Percino, J.
Synthesis, characterization, and optical properties of new two-photon- absorbing fluorine derivatives.
Chem.Materials **2004**, *16*, 4634-4641.

70 Benhida, R.; Lecubin, F.; Fourrey, J-L.; Castellanos, L.R.; Quintero, L.
Synthesis of 6-allyl- and 6-heteroarylindoles by palladiumcatalyzedStille cross-coupling reaction.
*Tetrahedron Lett.***1999**, *40*, 5701-5703.

71 Egi, M.; Liebeskind, L.S.
Heteroaromatic thioether-organostannane cross-coupling.
Org. Lett. **2003**, *5*, 801-802.

72 Mourtas, S.; Gatos, D.; Barols, K.
Solid phase synthesis of benzothiazolyl compounds.
*Tetrahedron Lett.***2001**, *42*, 2201-2204.

73 Lee, C.L.; Lam, Y.; Lee, S-Y.
Solid-phase combinatorial synthesis of benzothiazole and 2,3-dihydro-[1,5]-benzothiazepine derivatives.
*Tetrahedron Lett.***2001**, *42*, 109-111.

74 Yokum, T.S.; Alsina, J.; Barany, G.
Solid-phase synthesis of heterocycles containing the 2-aminothiophenol moiety.
J. Comb. Chem. **2000**, *2*, 282-292.

75 Hioki, H.; Matsushita, K.; Kubo, M.; Harada, K.; Kodama, M.; Fukuyama, Y.
Solid-phase combinatorial synthesis of benzothiazoles, benzimidazoles, and benzoxazoles using a traceless linker.
Tetrahedron, **2007**, *63*, 11315-11324.

76 Lim, H-J.; Myung, D.; Lee, I.Y.C.; Jung, M.H.
Microwave-assisted synthesis of benzimidazoles, benzoxazoles, and benzothiazoles from resin-bound esters.
J. Comb. Chem. **2008**, *10*, 501-503.

77 Kumar, A.; Maurya, R.A.; Ahmad, P.
Diversity oriented synthesis of benzimidazole and benzoxa/(thia)zole libraries through polymer-supported hypervalent iodine reagent.
J. Comb. Chem. **2009**, *11*, 198-201.

78 Ehrenkaufer, R.E.;Potocki,J.F.; Jewett,D.M.;
Simple synthesis of F-18-labeled 2-fluoro-2-deoxy-D-glucose: concise communication,
J. Nucl. Med. **1984**,*25*,333–337.

79 Teare, H.; Robins, E.G.;Arstad, E.;Luthra, S.K.;Gouverneur, V.;
Synthesis andreactivity of [F]–fluorobenzenesulfonimide,
Chem. Commun. **2007**, 2330–2332.

80 Dolbier, W.R.Jr.; Li,A.R.; Koch,C.J.;Shiue,C.Y.;Kachur,A.V.;
[^{18}F]-EF5, a marker forPET detection of hypoxia: synthesis of precursor and a new fluorination procedure
*Appl. Radiat. Isot.***2001**,*54*, 73–80.

81 Visser,G.W.M.;Gorree,G.C.M.;Braakhuis, B.J.M.; Herscheid,J.D.M.;
An optimizedsynthesis of [^{18}F]-labelled 5-fluorouracil and a reevaluation of its use as a prognostic agent,
Eur. J. Nucl. Med. Mol. Imaging,**1989**,*15*, 225–229.

82 Li,Z.; Conti,P. S.
Radiopharmaceutical chemistry for positron emission tomography
Adv. Drug Delivery Rev . , **2010**,*62*, 1031–1051

83 Reed, C. D.; Launay,G. G.; Carroll, M. A.;
Evaluation of tetraethylammonium bicarbonate as a phase-transfer agent in the formation of [^{18}F]fluoroarenes
*J. Fluorine Chem.***2012**,*143*, 231–237

84 Serdons,K.;Verduyckt,T.;Vanderghinste,D.;Cleynhens,J.;Borghgraef,P.;Vermaelen,P.;Terwinghe,C.; Van Leuven, F.; Van Laere,K.; Kung,H.;Bormans,G.;Verbruggen,A.
Synthesis of [^{18}F]-labelled 2-(4′-fluorophenyl)-1,3-benzothiazole and evaluation as amyloid imaging agent in comparison with [^{11}C]PIB
Bioorg. Med. Chem. Lett. **2009**,*19*, 602–605

85 Ryzhikov,N.N.;
Seneca,N.;Krasikova,R.N.;Gomzina,N.A.;Shchukin,E.;Fedorova,O.S.;Vassiliev,D.A.;Gulyas,B.;
Hall,H.;Savic,I.;Halldin,C.;
Preparation ofhighly specific radioactivity [^{18}F]-flumazenil and its evaluation in cynomolgus monkey by positron emission tomography,
Nucl. Med. Biol,**2005**,*32*, 109–116.

86 Pike, V.W.;Aigbirhio,F.I.
Reactions of cyclotron-produced [^{18}F]-fluoride withdiaryliodonium salts-a novel single-step route to no-carrier-added [^{18}F]fluoroarenes,
J. Chem. Soc., Chem. Commun . .**1995,** 2215–2216.

87 Lee, B.C.; Kim, J.S.; Kim, B.S.; Son, J.Y.; Hong, S.K.; Park, H.S.; Moon, B.S.; Jung, J.H.;, Jeong, J.M.; Kim, S.E.
Aromatic radiofluorination and biological evaluation of 2-aryl-6-[^{18}F]fluorobenzothiazoles as a potential positron emission tomography imaging probe for β-amyloid plaques
*Bioorg. Med. Chem.,***2011**,*19*, 2980–2990

88 Weekes, A. A.; Frydrych, J.; Westwell, A. D.;
Convenient Synthesis of Substituted 2-Phenylbenzothiazoles Using Solid-Supported Triphenylphosphine
. *Synth Comm* ,**2013** – in press

89 Yang,Z.; Chen,X.; Wang,S.; Liu,J.;Xie,K.; Wang,A.; Tan,Z.
J. Org. Chem. **2012** ,*77* , 7086-7091

90. Rahul,V. P.;Paresh, K. P.;Premlata, K.;Dhanji, P. R.;Kishor H. C.
Synthesis of benzimidazolyl-1,3,4-oxadiazol-2ylthio-*N*-phenyl (benzothiazolyl) acetamides as antibacterial, antifungal and antituberculosis agents
Eur. J. Med. Chem., **2012**,53, 41–51

91. Rana, A.; Siddiqui, N.; Khan, S.A.; EhtaishamulHaque S.; Bhat ,M.A.
N-{[(6-Substituted-1,3-benzothiazole-2-yl)amino]carbonothioyl}-2/4-substituted benzamides: Synthesis and pharmacologicalevaluation
Eur. J. Med. Chem., **2008**,*43*, 1114–1122

92 Jimonet, P;Audiau, F.;Barreau, M.;Blanchard, J.C ;Boireau, A.; Bour, Y.;Coléno,M.A.;Doble, A.;Doerflinger, G.;Huu,C.D.;Donat,M.H.;Duchesne,J.M.;Ganil, P.;Guérémy, C.; Honor, E.; Just, B.; Kerphirique, R.;Gontier, S.; Hubert, P.;Laduron, P.M.;LeBlevec, J.;Meunier, M.;Miquet,J.M.;Nemecek, C.;Mignani, S.; *et al.*
Riluzole Series. Synthesis and *in vivo* "Antiglutamate" Activity of 6-Substituted-2-benzothiazolamines and 3-Substituted-2-imino-benzothiazolines
*J. Med. Chem.***1999**,*42*,2828-2843.

93. Holla, B.S.; Malini, K.V.; Rao, B.S; Sarojini, B.K.; Kumari, N.S.
Synthesis of somenew2,4-disubstitutedthiazoles as possibleantibacterial and anti-inflammatory agents
Eur. J. Med. Chem. **2003**,*38*, 313–318

94. Kunzek, H.; Barnikow,
Isothiocyanate, XVII. Reaktionen von N-Aryl-α-cyan-malonsäure-äthylester-thioamiden
*G. Chem. Ber.***1969**, *102*, 351

95. Hiller,A.;Patt,J. T.; Steinbach, J.
On the reactionof 4-substitutedtrimethyltinaromatics with perchlorylfluoride
J. Organomet. Chem., **2006**,691, 3737–3742