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A Patent Review on Thiazole Derivatives (2008-2013)

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Abstract

Thiazole is a well-known five-membered heterocyclic compound. Various methods have been worked out for its synthesis. In the last few decades, a lot of work has been done on thiazole ring to synthetize derivatives directed to a plethora of molecular targets in order to discover new drug leads. The present review gives an account of the therapeutic patent literature (2008-2013) describing the applications of thiazole and its derivatives on selected activities. Most of the described compounds are shown to have potential beneficial therapeutic effects, even if all these patents generated few clinical evaluations. However, the thiazole scaffold remains one of great potential for the chemical pharmaceutical research; it can serve for the design of lead compounds especially in cases where the target is known and the mechanism of action is well defined.

Keywords:* *Enzymes; Inhibitor; Antitumor activity; Modulator; Thiazole

Introduction

Thiazole is a well-known five-membered heterocyclic compound. Various methods have been developed for its synthesis. In the last few decades, a lot of work has been done on thiazole ring to synthetize derivatives directed to a plethora of molecular targets in order to discover new drug leads.

The present review gives an account of the therapeutic patent literature (2008-2013) describing the applications of thiazole and its derivatives on selected activities. For the literature search, the Espacenet and the SciFinder databases for patent search have been used, applying essentially the keyword “thiazole” and the range from 2008 to 2013; while for articles related to patents Web of Science and PubMed bibliographic databases have been used. Using this kind of literature search, more than 200 patents inherent to molecules containing thiazole nucleus have been found. Only patents of pharmaceutical interest have been selected, for the discussion, by eliminating those linked to other research areas such as those claimed discoveries on agrochemical or petrochemical fields. Furthermore, the patents that describe compounds in which the thiazole ring is shown as a possible substituent in a more complex framework of a series, were not considered. At least one molecule of each patent examined was chosen by evaluating the biological data reported and the possible future therapeutic applications. Because of

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the huge amount of patents registered in this period, first, the attention was focused, in a review on inhibitors of protein kinases and on derivatives modulating enzymes related to metabolism [1]; then, in another review on thiazole derivatives having pharmacological activity towards receptors [2]. In this one we report a survey on: - thiazole derivatives having activity towards specific enzymes (endothelial nitric oxide synthase, pro-matrix metalloproteinase, prolyl hydroxylase, vascular adhesion protein-1, dipeptidyl peptidase-1, glycosidase, poly(ADP-ribose)polymerase), - thiazole derivatives with potential antitumor activity (inhibitors of: signal transducer and activator of transcription 3, murine double minute 2, vascular endothelial growth factor receptor 2, Traf2- and Nck-interacting kinase; targeting cells defective in the von Hippel-Lindau (VHL) gene, BH3-only protein mimetic; modulators of: Hec1 activity, estrogen-related receptor-α); - thiazole derivatives showing miscellaneous activity such as antiviral, anti-inflammatory, anti-mycobacterial, agonist of the TGR5 receptor, detrusor muscle-contracting, urethral sphincter muscle-relaxing as well as sodium channel or T-type calcium channel inhibitory activity, sirtuin or store-operated calcium channels modulatory activity, and for treatment of Duchenne muscular dystrophy. Most of the described compounds are shown to have potential beneficial therapeutic effects, even if all these patents generated few clinical evaluations. However, the high number of patents published, in the range of years considered (2008-2013), proves that the 1,3-thiazole scaffold, a small azole heterocycle, has received much attention from both industry and academic pharmaceutical researchers. In any case the thiazole scaffold has not exhausted its potential for research in pharmaceutical chemistry; it can still serve for the design of lead compounds especially if the biological target is known and the mechanism of action is well defined.

Thiazole derivatives modulating specific enzymes

Modulators of transcription for endothelial nitric oxide synthase

Endothelial NO synthase (eNOS, NOS-III) belongs to a group of three isoenzymes which produce nitric oxide (nitrogen monoxide, NO) by oxidation of arginine. Endothelial released NO is of central importance in a number of key cardiovascular mechanisms. It has a vasodilating effect and inhibits the aggregation of platelets, the adhesion of leukocytes to the endothelium and the proliferation of intimal smooth muscle cells [3]. The current assumption is that the transcriptional and post-transcriptional mechanisms of eNOS regulation are seriously disturbed in a large number of disorders, especially in cardiovascular disorders [4]. Some low molecular weight compounds which, in cell cultures, may lead to a direct effect on eNOS transcription and expression are disclosed in the literature [5,6]. In 2008 Zoller *et al.* claimed an application of derivatives of imidazo[2,1-*b*]thiazoles which modulate the transcription of eNOS and are valuable pharmacologically active compounds. Specifically, the new compounds up regulate the expression of the eNOS and can be applied in conditions in which an increased expression of the enzyme or an increased NO level or the normalization of a decreased NO level is desired. The compounds are useful for the treatment of various cardiovascular disorders. The determination of the biological activity includes the following assays: activation of eNOS transcription, anti-hypertensive effect in ApoE knockout mice and prevention of atherosclerotic plaque formation in chronic treatment and improvement of coronary function in diseased ApoE deficient mice. Several compounds of the invention, e.g. compound **1** (**FIG 1**), expressed EC_{50} values below 0.5 µM in an assay for activation of eNOS transcription [7].

Inhibitors of pro-matrix metalloproteinase activation

Matrix metalloproteinases (MMPs) are a family of structurally related zinc-dependent proteolytic enzymes that digest extracellular matrix proteins such as collagen, elastin, laminin and fibronectin. Currently, at least 28 different mammalian MMP proteins have been identified and they are grouped based on substrate specificity and domain structure. Enzymatic activities of the MMPs are precisely controlled, not only by their gene expression in various cell types, but also by activation of their inactive zymogen precursors (proMMPs) and inhibition by endogenous inhibitors and tissue inhibitors of metalloproteinases (TIMPs). A role for MMPs in oncology is well established [8], for instance MMPs appear to have a direct role in angiogenesis [9]. Other MMPs mediated indications include the cartilage and bone degeneration that results in osteoarthritis and rheumatoid arthritis. The degeneration is due primarily to MMPs digestion of the extracellular matrix (ECM) in bone and joints [10]. Various MMPs, including MMP9 and MMP13 have been found to be elevated in the tissues and body fluids surrounding the damaged areas. Elevated levels of MMP9 and MMP13 are also believed to be involved in atherosclerotic plaque rupture, aneurysm and vascular and myocardial tissue morphogenesis [11-13]. The inactive form of MMP9, proMMP9, is expressed with several different domains [14-18]. Based on the demonstrated involvement in numerous pathological conditions, inhibitors of MMPs have therapeutic potential in a range of disease states [19]. However, non-selective active site MMPs inhibitors have performed poorly in clinical trials. The failures have often been caused by dose-limiting toxicity and the manifestation of significant side effects, including the development of musculoskeletal syndrome (MSS). It has been suggested that development of more selective MMPs inhibitors might help to overcome some of the problems that hindered clinical success in the past. MMPs share a catalytically important Zn^{2+} ion in the active site and a highly conserved zinc-binding motif. In addition, there is considerable sequence conservation across the entire catalytic domain for members of the MMPs family. A novel approach to develop more selective MMPs inhibitors is to target the pro-domain of the inactive zymogens, proMMPs, with allosteric small molecule inhibitors that bind and stabilize the inactive pro-form of the protein and inhibit processing to the active enzyme. There is significantly less sequence identity within the pro-domains of MMP proteins, no catalytically important Zn^{2+} ion, and no highly conserved zinc-binding motif. Thus targeting the pro-domain of proMMPs is an attractive mechanism of action for inhibiting the activity of the MMP proteins [20-21]. There are no reports, however, of allosteric small-molecule inhibitors that bind the pro domain and inhibit activation of proMMP9, proMMP13, or any other proMMP. In 2012, Janssen Pharmaceutical NV patented the preparation of tricyclic inhibitors of proMMP activation [22]. This invention relates to tricyclic compounds with an (un)substituted ring such as thiazole, imidazole, triazole, pyridine. Over one-hundred-sixty compounds were prepared by a multi-step synthesis, starting from 1,2-phenylenediamine and benzoylisothiocyanate. They were tested in the Thermo Fluor® (TF) binding assays against proMMP9 and proMMP13 that measures thermal stability of proteins [23,24] and data are given. In these binding assays compound 2 (**FIG** 1) had Kd=3,6 µM using the protein proMMP9(20-445;ΔFnII)(SEQ ID NO:6) and Kd=7 µM with the protein proMMP13(1-268)(SEQ ID NO:7). In the same year, Janssen recorded another series of patents that are described below annotating with the first name of the inventor. Zhang et al. patented novel pyridyl-thiazolyl derivatives as small-molecule allosteric inhibitors of the proteolytic activation of proMMP9 and proMMP13 [25]. These molecules may have therapeutic uses in inflammation disorders or in disorders ameliorated by inhibiting the proteolytic activation of proMMPs. Considering all the derivatives described in the patents, compound 3 (**FIG 1)** resulted the most active in inhibiting the proMM9 activation with an IC50 of 0.059 µM whereas compound 4 (**FIG 1)** was the most active in inhibiting the proMM13 activation (IC50 of 0.90 µM). Leonard et al. disclosed an invention related to fused heteroaryl inhibitors of pro-MMPs activation for preventing, treating or ameliorating MMP9 or MMP13 mediate diseases and associated with MMP9 or MMP13

overexpression [26]. Researchers report the synthesis of new thiazoles carried out according to the classical scheme of the condensation reaction between bromobenzophenones suitably substituted and alkyl or aryl thiourea. In the Thermo Fluor® (TF) binding assays against proMMP9 and proMMP13 compound 5 (**FIG 1)** had Kd=1,2 μM when the protein proMMP9(20-445;ΔFnII)(SEQ ID NO:6) was used and Kd=1,9 μM with the protein proMMP13(1-268)(SEQ ID NO:7). Jackson et al. claimed some related patent applications of phenyl-thiazolyl or bisthiazole derivatives as proMMPs inhibitors [27-30]. The activity of the compounds was studied carefully by performing the assays of enzyme activation, cell assays and in vivo experiments. For example, in the Thermo Fluor® (TF) binding assays compound 6, 3-(2',4'-dimethyl-[4,5']bithiazolyl-2-ylamino)-4-isopropoxy-benzensulfonamide (**FIG 1**), had Kd = 0,10 μM against proMMP9 and Kd = 0,14 μM against proMMP13. Furthermore, the above mentioned patents claimed that this compound blocked production of active MMP9 by rat synoviocytes with an IC50 of 1.1 μM, as well as in human fetal lung fibroblasts (HFL-1, IC_{50} of 0.3 μM). Finally, the patents reported in vivo studies performed on compound 6 which demonstrated that expression and activation of proMMP9 is increased in rat with streptococcal cell wall (SCW)-arthritis. Afterwards they claimed that treatment of rats with SCW-induced arthritis by compound 6 reduced active MMP9 in ankles, induced a dose-dependent decrease in ankle thickness and, in the liver, reduced MMP9 mediated gelatinase activity.

Inhibitors of prolyl hydroxylase activity

The cellular transcription factor HIF (Hypoxia Inducible Factor) occupies a central position in oxygen homeostasis in a wide range of organisms and is a key regulator of responses to hypoxia [31,32]. The HIF transcriptional complex is a heterodimer formed by HIF-α, a constitutive nuclear protein that dimerizes with oxygen-regulated HIF-α subunits. Oxygen regulation occurs through hydroxylation of the HIF-α subunits, which are then rapidly destroyed by the proteasome [33,34]. In 2008 Allen et al. patented the preparation of approximately thirty thiazolepyridine-based compounds exhibiting prolyl hydroxylase inhibitory activity. Compounds of the invention may exist in multiple tautomeric forms. The invention relates to compounds able to inhibit prolyl hydroxylases such as Hypoxia Inducible Factor Prolyl hydroxylases (HIF PHD) or to modulate HIF levels or to stabilize HIF. The compounds and compositions may be used to treat diseases or conditions modulated by HIF such as ischemia, anemia, wound healing, auto-transplantation, allo-transplantation, xeno-transplantation, systemic high blood pressure, thalassemia, diabetes, cancer, and inflammatory disorders. Newly synthesized thiazolepyridine were assayed by enzymatic binding tests to measure their inhibitory activity toward HIF PHD. They exhibited a HIF PHD inhibitory activity IC_{50} value of 40 μM or less. Compound 7 (**FIG 1**) showed IC_{50} of 0.020 μM for inhibition of HIF PHD [35].

Inhibitors of vascular adhesion protein 1

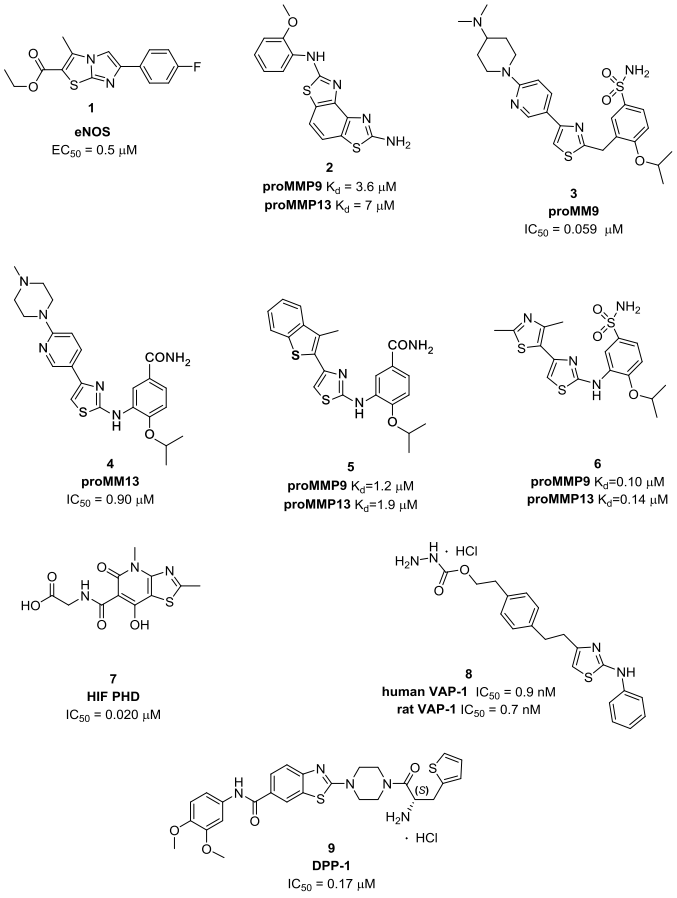
The vascular adhesion protein-1 (VAP-1) is an amine oxidase, also known as semicarbazide sensitive amine oxidase (SSAO), abundantly existing in human plasma, which shows a remarkably increased expression in vascular endothelium and vascular smooth muscle in the inflammatory lesion. Reports have demonstrated that VAP-1 enzyme activity in plasma increases both in type I and type II diabetic patients [36]. Some patents [37,38] describe thiazole derivatives having specific structures and that they can be used for the prophylaxis or treatment of VAP-1 associated disease such as macular edema, vascular hyper permeable disease and the like. In these patents the thiazole derivatives have a specific framework encompassing a hydrazino group or a hydrazinocarbonyl group at the molecular terminal. In 2008, Kawai [39] reported the synthesis of acylaminothiazole derivatives as VAP-1 inhibitors. The invention aims to provide new thiazole derivative VAP-1 inhibitors useful for the prophylaxis or treatment of VAP-1 associated diseases. As a result of intensive studies, the inventors have

found that thiazole derivatives having a specific functional group (carbazic acid ester or carbazic acid thioester or semicarbazide group) at the molecular terminal have superior VAP-1 inhibitory action, superior enzyme selectivity and less side effects. The compounds were examined for the enzyme activity inhibitory effect on human and rat VAP-1 enzyme. Compound 8 (**FIG 1**) showed IC_{50} of 0.9 and 0.7 nM against human and rat VAP-1 enzyme respectively [39].

Inhibitors of dipeptidyl peptidase-1

Dipeptidyl peptidase-1 (DPP-1, cathepsin C) is a member of the lysosomal papain-type cysteine protease family that also includes cathepsin B, K, H, L, O, and S. DPP-1 (MW 200 kd) is composed of a dimer of disulfide-linked heavy and light chains, both from a single protein precursor [40, 41]. The biological function of DPP-1 is to convert inactive proenzymes into active enzyme by removing a dipeptide from N-terminal. Since these enzymes play an important pathological role in Chronic Obstructive Pulmonary Disease (COPD), inhibition of DDP-1 by small molecules would be a rational therapeutic intervention for COPD. Additional therapeutic indications for a DPP-1 inhibitor are asthma, rhinitis, and rheumatoid arthritis [42]. In 2011 Janssen Pharmaceutical patented substituted benzothiazole and benzoxazole derivatives inhibitors of DPP-1. Test compounds were assessed for DPP-1 inhibitory activity using a fluorogenic substrate, GR-AMC (Glycine-Arginine amino-4-methylcoumarin, Bachem, 1-1215). The amount of amino-methylcoumarin released is proportional to the DPP-1 activity. Compound **9** (**FIG 1**), prepared in a 4-step reaction scheme that involved reaction of Boc-(2-thienyl)-L-alanine and N-(3,4-dimethoxyphenyl)-2-(piperazin-1-yl)benzol[d]thiazole-6-carboxamide and deprotection of the intermediate formed, is the most active of the whole series with IC_{50} 0.17 µM [43].

FIG. 1**. Thiazole derivatives modulating enzymes**



Selective inhibitors of glycosidase

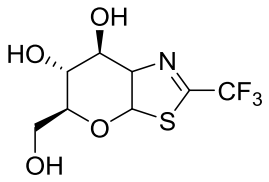
A wide range of cellular proteins, both nuclear and cytoplasmic, are post-translationally modified by the addition of the monosaccharide 2-acetamido-2-deoxy-D-glucopyranoside (N-acetylglucosamine) which is attached via a glycosidic linkage [44], generally referred to as O-linked N-acetylglucosamine or O-GlcNAc. The enzymes involved in removal of these sugars are N-acetyl-beta-D-glucosaminidases (O-GlcNAcase). As a result of the biological importance of these O-GlcNAcases, small molecule inhibitors of glycosidases have received a great deal of attention, both as tools for elucidating the role of these enzymes in biological processes and in developing potential therapeutic applications [45,46]. A major challenge in developing inhibitors for blocking the function of mammalian glycosidases is the large number of functionally related enzymes present in tissues of higher eukaryotes [47]. A few of the better characterized inhibitors of N-acetyl-glucosaminidases which have been used in studies of O-GlcNAc post-translational modification within both cells and tissues are streptozotocin (STZ), 2'-methyl-α-D-glucopyrano[2,1-d]Δ2'-thiazoline (NAG-thiazoline) and O-(2-acetamido-2-deoxy-D-glucopyranosylidene)amino-N-phenylcarbamate (PUGNAc). STZ has long been used as a diabetogenic compound because it has a particularly detrimental effect on islet cells [48]. In 2008 Vocadlo *et al.* patented the synthesis of pyrano[3,2-d]thiazole which selectively inhibit glycosidases [49]. The invention also comprises animal models for studying disease and disorders related to deficiency or overexpression of O-GlcNAcase, accumulation or deficiency of O-GlcNAc. A logical starting point for the design of inhibitors takes into consideration the catalytic mechanism of an O-GlcNAcase and β-hexosaminidase. Although the catalytic mechanism of action of two dimeric isozyme β-hexosamimidase A and B, members of family 20 of glycoside hydrolase, have been established, that of the family 84 O-GlcNAcase was unknown [50]. The inventors first elucidate the catalytic mechanism of human O-GlcNAcase and secondly, used this information in designing simple inhibitors that would be potent, cell permeable and highly selective [51]. This allowed the synthesis of a first series of inhibitors in which the thiazoline ring was elaborate with aliphatic chains of increasing length in the expectation that these compounds would allow the discriminative inhibition of O-GlcNAcase over lysosomal hexaminidase. Two synthetic route enable the production of quantities of inhibitors from commercially available starting materials in three steps or from the inexpensive starting material 2-amino-2-deoxy-glucopyranose in six steps. Based on results from kinetic analyses using thiazoline derivatives, the inventors designed and synthesised a second series of inhibitors modifing the PUGNAc scaffold. In the biological assayes the pyranothiazole derivative **10** (**FIG 2**) shown a O-GlcNAcase K_i = 5.6 µM. When tested in the assay for determination of K_i values for inhibition of β-hexosaminidase activity, many of the compounds described herein exhibit K_i values in the range 5 µM - 10 mM. In general, the compounds described exhibit a selectivity ratio in the range of about 1000 to 100000; where the selectivity ratio for inhibition of O-GlcNAcase over β-hexosaminidase is define as: K_i (β-hexosaminidase/K*i* (O-GlcNAcase)). In 2012 Kaul *et al*. patented the preparation of another library of 2-amino-pyrano[3,2-d]thiazole derivatives useful as selective glycosidase inhibitors to treat disorders related to deficiency or over expression of O-GlcNAcase, and accumulation or deficiency of O-GlcNAc [52] such as neurodegenerative diseases for example Alzheimer disease (AD). In effect there is a large body of evidence indicating that in AD patients by inhibiting the action of O-GlcNAcase, one should be able to block hyperphosphorylation of tau and all of the associated effects of tau hyperphosphorylation, including the formation of neurofibrillary tangles and downstream effects [53,54]. Compounds were tested for determination of K_i values for inhibition of O-GlcNAcase activity. The amount of purified human O-GlcNAcase enzyme used in the reaction is 0.7 nM. K_i values are included in a range of 0.3-164 µM, i.e. product **11** (**FIG 2**) shown a K_i =

0.50 μM. In 2013, Donnelly [55] described the preparation of about a hundred of pyranol [3,2-d][1.3]thiazoles as glycosidase inhibitors. In particular the compounds of the invention may be present as pure E or Z isomers, or in the form of mixture of these isomers [55]. Moreover the compounds have asymmetric center and, consequently, they can exist in the form of racemates, of pure enantiomers and/or diastereoisomers or in the form of mixtures. Authors claimed that the mixtures of new derivatives were separated and purified according to the classical chemical methods when possible before testing. They were tested by an fluorescent and direct human O-GlcNAcase enzyme inhibition assay and by an immunoadsorbant assay (ELISA) technique for the determination of activity of the same enzyme on cellular protein, i.e. compound **12** (**FIG 2**) showed in the first and in the second assay a value of IC_{50} < 0.1 μM and 0.1-0.5 μM respectively.

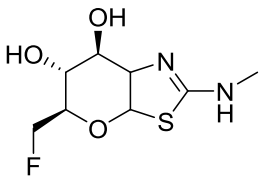
Modulators of poly(ADP-ribose)polymerase

The poly(ADP-ribose)polymerase (PARP) family, consisting of at least five members, catalyzes the post translational modification of several nuclear proteins in response to DNA damage. PARP activation is involved in the ability of cells to repair injured DNA. Because of PARP's role in DNA repair a number of PARP inhibitors are being currently developed clinically or are already in clinical trials for the treatment of various diseases and conditions, including chronic and acute neurological and cardiovascular conditions and cancers [56-58]. In 2009 Pellicciari et al. reported the preparation of thiazolyl-isoquinolinones as therapeutic poly (ADP-ribose) polymerase modulators. The invention also relates their use in inhibiting neuronal cell death and treating tissue damage due to ischemia and reperfusion, degenerative diseases, inflammatory diseases, and cancer. A multi-step synthesis of derivative 13 (**FIG 2**), which showed an IC_{50} of 378 nM against human PARP-1 in functional assessment of human PARP-1 enzymatic activity, was given [59].

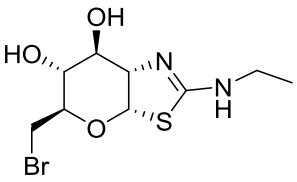
FIG. 2. **Thiazole derivatives modulating enzymes**



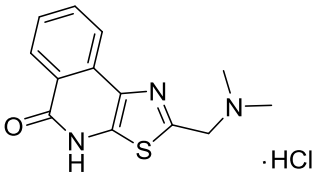
10
O-GlcNAcase K_i = 5.6 μM



11
O-GlcNAcase
K_i = 0.50 μM



12
O-GlcNAcase IC_{50} < 0.1 μM
ELISA IC_{50} = 0.1-0.5 μM



13
human PARP-1
IC_{50} = 378 nM

Thiazole derivatives with potential antitumor activity

Inhibitors of signal transducer and activator of transcription 3

As the name implies, signal transducer and activator of transcription 3 **(**STAT3), is a transcription factor and, in humans, it is encoded by the gene appropriately known as the *STAT3* gene [60,61]. Accordingly, STAT3 has a central role in important cellular processes involving cell growth and death, apoptosis. Presence of constitutive STAT3 is found in a number of human cancers [62,63]. STAT proteins mediate the relay of extracellular signals from various cell surface protein receptors to the nucleus, where they help to initiate and regulate specific anti-apoptotic and cell survival gene expression. Successful peptidic and non peptido-mimetic small molecules, that are capable of targeting malignant cell lines with constitutively activated STAT3 protein, are limited to a few examples which were all identified through high-throughput virtual or biochemical screening approaches [64]. The first generation designs were simple peptidomimetics derived from the natural sequence, of which ISS-610 was the most potent [65]. An oxazole-based small-molecule inhibitor, S31-M2001 that shows promising inhibition of STAT3 function, was recently discovered [66]. This agent inhibits STAT3 protein dimerization and induces apoptosis in STAT3-transformed cells and STAT3-dependent breast oncogenic cell lines. In 2010 Turkson *et al.* claimed the preparation of thiazole derivatives as inhibitors of transcription factor STAT3. The invention discloses various small molecules, which are inhibitors of STAT3. Additionally, the invention includes compositions containing the disclosed STAT3 inhibitory compounds, as well as methods of treatment employing these compounds and compositions. The investigation sought to rationally develop small molecule probes derived from the native STAT3 binding sequence. The patent reports the rational design and synthesis of small molecule STAT3 inhibitors which selectively inhibit STAT3 protein dimerization and induce apoptosis in STAT3 transformed cells and STAT3-dependent breast and pancreatic oncogenic cell lines. The crystal structure of the STAT3-SH2 domain (Src Homology 2: SH2) includes a shallow triangular pocket containing two hydrophobic sites and a hydrophilic phosphate-recognition pocket. Docking studies of the initial lead compound, peptidomimetic ISS-610, indicated that only the hydrophobic pockets in the binding domain were effectively occupied. Therefore, it was theorized that trisubstituted heterocyclic scaffolds, such as oxazoles and thiazoles, might effectively access all three sites. Flexible ligand-docking studies directed the design and assembly of oxazole and thiazole scaffolds. The disclosed compounds demonstrate inhibitory activity against STAT3. E.g., thiazole derivative **14** (**FIG. 3**) exhibited IC_{50} value of 33 μM for the inhibition of STAT3 DNA-binding activity *in vitro*. Suitably substituted oxazole and thiazole scaffolds, derived from peptidomimetic leads, disrupted STAT3:STAT3-DNA-binding activity *in vitro* at low micromolar concentrations, but showed low affinity to the unphosphorylated STAT3 monomer. This might suggest that the present compounds preferentially bind with activated STAT3, a hypothesis which is, however, to be verified. However, lead agents showed potency and specific human cancer cell lines and negligible toxicity towards normal NIH-3T3 fibroblasts. These studies highlight the widely acknowledged belief that STAT3 is a potent target for small-molecule inhibitors in the development of novel anticancer drugs and that targeting of STAT3 will require yet a further level of investigation [67].

Targeting cells defective in the von Hippel-Lindau (VHL) gene

Mutations and/or inactivation of the von Hippel-Lindau (VHL) tumor suppressor gene occur in the majority of clear cell renal carcinoma (CC-RCC) and is associated with metastatic disease [68]. Functional studies indicate that pVHL, the protein product of VHL, is an ubiquitin ligase that targets the α-subunit of the hypoxia-inducible factor (HIF) for proteasomal degradation under normoxia. In the presence of oxygen, hydroxylation on proline residues 564 and 402 by prolyl hydroxylases marks HIF-α for recognition and binding with pVHL, leading to degradation of HIF- α. Under hypoxic conditions, activity of the prolyl hydroxylases decrease, which prevents the recognition of HIF- α by pVHL [69,70]. In cells that lack VHL, stabilized HIF- α binds to activate the transcription of genes involved in several processes [71-75]. In 2011 Turcotte *et al*. patented the preparation of pyridinyl-substituted-1,3-thiazol-2-amine derivatives useful for the treatment of cancer. The researchers previously published the biological activity profile of a small molecule, STF-62247, a pyridinyl-thiazol-2-amine derivative, included in the patent, that selectively induces cell death in VHL-deficient cells and that represents a paradigm shift for targeted therapy. STF-62247 shows selective toxicity and growth inhibition of renal cells lacking VHL; 25-fold greater sensitivity was observed for cells with VHL deficiency compared to wild-type (VHL+). Currently STF-62247 is on clinical trials phase II (NCT01829321, NCT01779466) [76,77]. The patent provides novel heteroaryl compounds, compositions, and methods of use in targeting cells defective in the von Hippel-Lindau (VHL) gene and diseases associated with such defects. Based on the STF-62247 structure, other compounds were designed and synthesized to evaluate their ability to induce selective death in VHL-deficient cells. Analysis of the high throughput screening data indicated the importance of a 4-pyridyl unit linked to the 4-position of the thiazole, while the tested 3-pyridyl analogs were inactive. A thiazole with an amino group was fundamental for activity suggesting an H-bond contact in this region. Substituents at the 2-position on the aryl ring were not tolerated in the tested compounds. The compounds induce cytotoxicity and reduce tumor growth of VHL-deficient cells compared to genetically matched cells with wild-type VHL. The compounds thus selectively induce cell death in VHL-deficient cells and therefore represent a novel strategy for targeted therapy. *In vitro* tests for identification of molecules targeting VHL-deficient renal cell carcinoma as antitumor agents, STF-62247 (**15**, **FIG. 3**) exhibited IC_{50} value of 2.1 µM in renal carcinoma 4 (RCC4) cells [78].

BH3-only protein mimetic

Apoptosis is now recognized as an essential biological process in the tissue homeostasis of all living species. Several apoptotic pathways have been uncovered, and one of the most important involves the Bcl-2 family of proteins, which are key regulators of the mitochondrial (also called "intrinsic") pathway of apoptosis [79]. The structural homology domains BH1, BH2, BH3 and BH4 are characteristic of this family of proteins. The Bcl-2 family of proteins can be further classified into three subfamilies depending on how many of the homology domains each protein contains and on its biological activity (i.e., whether it has pro- or anti-apoptotic function). The third subgroup is composed of proteins containing only the BH3 domain and members of this subgroup are usually referred to as "BH3-only proteins." Their biological effect on the cell is pro-apoptotic. Bim, Bid, Bad, Bik, Noxa, Hrk, Bmf, and Puma are examples of this third subfamily of proteins [80-82]. There is a large body of scientific evidence to show that compounds, which inhibit the binding of BH3-only proteins to anti-apoptotic Bcl-2 family proteins, promote apoptosis in cells. In one aspect, the implication that down-regulated apoptosis (and more particularly the Bcl-2 family of proteins) is involved in the onset of cancerous malignancy has revealed a novel way of targeting this still elusive disease [83-85]. These findings as well as numerous others have made possible the emergence of new strategies in drug discovery for targeting cancer: if a small molecule that could mimic the effect of BH3-only proteins

was able to enter the cell and overcome the anti-apoptotic protein over-expression, then it could be possible to reset the apoptotic process [86]. This strategy can have the advantage of alleviating the problem of drug resistance, which is usually a consequence of apoptotic deregulation (abnormal survival). Abbott Laboratories Inc. has developed a class of small molecule BH3-only protein mimetics, *i.e*., ABT-737 and ABT-263 (Navitoclax) [87-90], that bind strongly to a subset of anti-apoptotic Bcl-2 proteins including Bcl-2, Bcl-w and Bcl-xD but only weakly to Mcl-1 and A1, and exhibit mechanism based on cytotoxicity. These compounds were tested in animal studies and demonstrated cytotoxic activity in certain xenograft models as single agents [91]. These *in vivo* studies suggest the potential utility of inhibitors of anti-apoptotic Bcl-2 family proteins for the treatment of diseases that involve a deregulated apoptotic pathway. In view of the important role for Bcl-2 family of proteins in regulating apoptosis in both cancerous and normal (i.e., non-cancerous) cells, and the recognized inter-cell type variability of Bcl-2 family protein expression, it is advantageous to have a small molecule inhibitor that selectively targets and preferably binds to one type or a subset of anti-apoptotic Bcl-2 protein(s), for example, to an anti-apoptotic Bcl-2 family member that overexpressed in a certain cancer type [92]. In 2009, Watson [93] patented the preparation of benzothiazole compounds as mimics of BH3 only proteins able to neutralize pro-survival Bcl-2 proteins [93]. The invention is predicated in part on the discovery that benzothiazole derivatives provide a BH3-only protein mimetic which is able to interact with a Bcl-2 protein. The patent also relates to processes to the use of such compounds in the treatment and/or prophylaxis of diseases or conditions associated with the deregulation of cell death or cell survival. In a competitive binding assay for a Bcl-2 binding site compound **16** (**FIG 3**) had an IC_{50} <10 µM. Measurement of the affinity of the compounds of the invention for Bcl-2 protein was also determined using a competitive binding assay based on fluorescence polarization (FP). For this assay fluorescein labelled Bak peptide, which is known to bind to the hydrophobic groove of Bcl-2 with high affinity, was used following the general method described by Wang [94]. Benzothiazole **16** showed IC_{50} value below 1 µM. In 2010 Baell *et al*. claimed an invention for the preparation of N-benzothiazolyltetrahydroquinolinecarboxamides useful in treatment of diseases characterized by the expression or overexpression of Bcl-2 antiapoptotic proteins. The measurement of competition of compounds for a Bcl-2 family protein (Bcl-xL) binding site was determined using a fluorescence binding assay. In these experiments benzotiazolecarboxamide derivative **17** (**FIG 3**) showed a K_i=0.018µM [95]. .

Inhibitors of murine double minute 2

Functional integrity of the p53 gene is an essential cellular defense against neoplastic transformation [96]. This is highlighted by the fact that 50% of human cancers have mutated p53. On genotoxic or nongenotoxic cellular stress, p53 is activated orchestrating several cell cycle and apoptotic regulatory pathways. Murine double minute 2 (MDM2; HDM2 in humans) is a master regulator of p53 [97, 98]. MDM2 inhibits p53 through three mechanisms: first, binding of MDM2 to the transactivation domain of p53, inhibits p53 transcriptional activity; second, binding of MDM2 to p53 facilitates its export from the nucleus toward proteasome degradation; and third, MDM2 acts as p53-ubiquitin ligase augmenting degradation by the proteasome and thus down-regulating p53 protein levels. Because almost half of human cancers have unmutated or wild type p53 gene, many efforts have been made in developing therapeutic agents that p53-binding pocket of MDM2 specifically activate p53. An increasing number of MDM2 antagonists are being generated and some of them have entered clinical trials [99]. One of this is the imidazoline derivative Nutlin-3A [100]. The active enantiomer of Nutlin-3A inhibits the p53/MDM2 interaction with IC_{50} of 90 nM [101]. In 2009, Kawato [102] patented the detailed synthesis of two hundred of 5,6-dihydroimidazo[2,1-b][1,3]thiazole derivatives structurally similar to Nutlin-3A, an imidazoline having two sites substituted

with halogenobenzene [102]. The products of this library inhibit the interaction between MDM2 protein and p53 and show an antitumor activity. Thus, the *cis* isomer dihydroimidazo[2,1-b][1,3]thiazole **18** (**FIG 3**) inhibited the p53/MDM2 interaction with IC_{50} of 10 nM and the proliferation of human lung cancer cell NCI-H460 and human colon cancer cell DLD-1 with GI_{50} of 2.81 and >50 μM.

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Modulators of Hec1 activity

Kinetochores are protein scaffolds coordinating the process of chromosome segregation in mitosis [103]. Hec1, a kinetochore outer layer component and spindle checkpoint regulator, is of particular interest because it clearly has association with cancer progression. Hec1 directly interacts with multiple kinetochore components, including Nuf2, Spc25, and Zwint-1, and with mitotic kinases Nek2 and Aurora B [104-106]. The primary functions of Hec1 are assigned to mitotic control for kinetochore assembly, K-fiber attachment, chromosome alignment, and the retaining of Mad2 spindle checkpoint protein to inhibit premature anaphase entry. Interestingly, overexpression of Hec1 was previously observed in a variety of human cancers and a large portion of the NCI-60 cancer lines [107], which was later found to be an excellent prognosis marker for primary breast cancers and patients with multiple cancers [108-110]. In 2013 Huang et al. describe some thiazoles derivatives for modulation of Hec1/Nek2 interaction [111]. The inventors have discovered that the compounds are capable of selectively disrupting a Hec1/Nek2 complex and/or to selectively preventing Hec1 from binding to Nek2. Consequently, the compounds are capable of inducing abnormal mitosis and apoptosis in cancer cells, and of accumulating sub-G1 apoptotic cells. More recently, various small molecule inhibitors, containing the thiazole scaffold and interfering with the Nek2/Hec1 interaction, have been developed [112]. Since Nek2 is a regulatory component of Hec1 in mitosis, abrogation of the Hec1/Nek2 function was expected to result in chromosome mis-aggregation. New compounds were tested to determinate the antiproliferative activity on selected cancer cells. For example, derivative 19 (**FIG 3**) showed the following antiproliferative effect: on HeLa IC50 = 21.1 nM; on K562 IC50 = 18 nM; on MDA-MB-468 IC50 = 19.1 nM; on MDA-MB-231 IC50 = 35.1 nM. Moreover, selected compounds of the invention were subject to a series of detailed biological and immunochemical assays. Co-immunoprecipitation (Co-IP)/Western blotting assays were performed to assess the effects of Hec1 inhibitory compounds on Hec1-Nek2 interaction: results show decreased Hec1 level in the Nek2 immunoprecipitates of cells treated with compound 19. Furthermore, for 19, DNA content analysis shows an increase in the sub-G1 population. This is indicative of DNA fragmentation during apoptosis, suggesting the induction of apoptosis by 19, which leads to drug-induced cell death. Studies of inhibition of xenograft tumor outgrowth by compound 19 on xenografted breast cancer models and a Huh-7 liver cancer model in nude mice show that despite the tumor reduction body weight remained constant in all cases, suggesting that overall toxicity of Hec1 inhibitory is low. Development of resistance to treatment in advanced stage tumors is a well-known phenomenon. Re-treatment with 19 demonstrates that the Hec1 inhibitor remained effective with late stage tumors. This suggests that such compounds may have a reduced tendency to induce resistance in tumors and other proliferative diseases. To determinate the Hec1 inhibitory binding to hERG of derivative 19, [3H]-astemizole competitive binding assays were performed. IC50 of tested drug was >10 μM.

Inhibitors of vascular endothelial growth factor receptor 2

Angiogenesis plays a central role in the process of tumor growth and metastatic dissemination. Agents directed either against vascular endothelial growth factor VEGF or VEGF receptors (VEGFRs) have been developed. The tyrosine kinase inhibitors of VEGFRs are low molecular weight, ATP-mimetic that bind to the ATP-binding catalytic site of the tyrosine kinase domain of VEGFRs, resulting in blockade of intracellular signaling. Large randomized phase III trials have demonstrated the efficacy of sunitinib and sorafenib in the treatment of patients affected by gastrointestinal stromal tumors and renal cancer refractory to standard therapies, respectively. For other agents, such as vatalanib, contrasting outcomes in metastatic colorectal cancer patients have been reported. However, several key questions remain to be addressed, regarding the choice of an adequate dose or schedule, the presence of "off-target" effects, the safety of long-term administration [113]. The vascular endothelial growth factor (VEGF) family of angiogenic growth factors includes six secreted glycoproteins [114-116]. VEGF ligands act through specific binding to three different cell membrane receptors [117]. In 2013, Beroza [118] claimed an invention relates to substituted thiazoles and their use as VEGFR2 kinase inhibitors [118]. The compounds of this disclosure are generally synthesized using published methods, but it is only apparent to a person of ordinary skill in the art, that methods to synthesize all derivatives are included in the examples of the claims. To investigate the cytotoxic/cytostatic effects about 70 derivatives are subject to the following tests: *in vitro* VEGFR2 kinase assay, *in vitro* HL-60 (human promyelocytic leukemia) assays and HCT 116 (human colon carcinoma) cytotoxicity assays and only for selected compounds, *in vivo* HCT116 xenograft model. Compound **20** (**FIG 3**) shows these results: 10 nM (VEGFR2 kinase assay), 1 µM (HL-60 cytotoxicity assays) and 0.3 µM (HCT-116 cytotoxicity assays). Moreover, it was active in the *in vivo* assay and showed inhibition of tumor growth compared to vehicle.

Modulators of estrogen-related receptor-α

Estrogen-related receptor-α (ERR-α, NR3B1) belongs to the estrogen-related receptor subfamily (ERR subfamily: orphan nuclear receptor closely related to ER). ER and the ERR subfamily are known to have common target genes such as estrogen-responsive genes [119-121]. In consideration of the wide activity of ERR-α, an ERR-α modulator is expected to be useful for the prophylaxis or treatment of various disease conditions [122,123]. ERR-α has attracted attention as a therapeutic target in triple-negative breast cancers with de novo resistance to, and in breast cancers with acquired resistance to, endocrine therapies such as antiestrogens and aromatase inhibitors [124,125]. In 2013, Shigemitsu Matsumoto [126] claimed an invention provides some 1,3-thiazol-2(5H)-one derivatives having a superior activity as an ERR-α modulator [126]. Since the compounds of the present invention has a superior activity as an ERR-α modulator (particularly, inverse agonist), they could be useful as agents for the prophylaxis or treatment of ERR-α associated diseases such as malignant tumor (e.g. malignant lymphoma, multiple myeloma, cancer of breast, prostate, colon, lung, ovarian cancer, endometrial carcinoma). The compounds were prepared according standard chemistry methods. The inverse agonist activity of the compounds was evaluated in HTRF assay system using ERR-α ligand-binding domain by a fluorescence method. The results were reported as percentage rate of activity at the concentration of the modulators of 30 µM; considering a value of inhibition as 0% with no addition of compound. Derivative 21 (**FIG 3**) showed an inhibition rate of 100. Some selected compounds were subjected to an antitumor test using MDA-MB-231-luc cells transplanted subcutaneously in mice. In the experiments, setting the change in tumor volume of the vehicle-treated group as 100%, the change rate in tumor volume of the compound-treated group (T/C %) was calculated. Compound 21 at the 50 mg/kg dose for 14 days showed T/C % = 24 and therefor an appreciable antitumor effect.

Inhibitors of Traf2- and Nck-interacting kinase

In 2013, Masaaki Sawa et al. claimed an invention relates to novel bicyclic thiazole compounds that inhibit Traf2- and Nck-interacting kinase (TNIK) [127]. The inventors describe that the new derivatives are useful as TNIK inhibitors administered to cancer patients, especially solid cancer such as colorectal cancer, pancreatic cancer, non-small cell lung cancer, prostate cancer or breast cancer. TNIK is known as one of STE20 family kinases that activates the c-Jun N-terminal kinase pathway and regulates the cytoskeleton [128,129]. Recently, TNIK was identified as one of 70 proteins immunoprecipitated commonly with anti-TCF4 (T-cell factor-4) and anti-β-catenin antibodies in two colorectal cancer cell lines DLD1 and HCT-116 [130]. Recent studies has been shown that TNIK plays critical roles in canonical Wnt signaling pathway, and therefore TNIK can be a promising target to ablate aberrant Wnt signaling in tumors [131]. The bicyclic thiazole compounds show the TNIK inhibitory effects and a remarkable antiproliferative activity. The TNIK inhibitory effects were determined in a kinase assay using recombinant Human TNIK (N-Terminal Segment), while for in vitro cell proliferation assay; the human colon cancer cell line HCT-116 was used. Compound 22 (**FIG 3)** showed an IC50 = 8.0 nM in the TNIK test and an IC_{50} < 100 nM in the HCT-116 test.

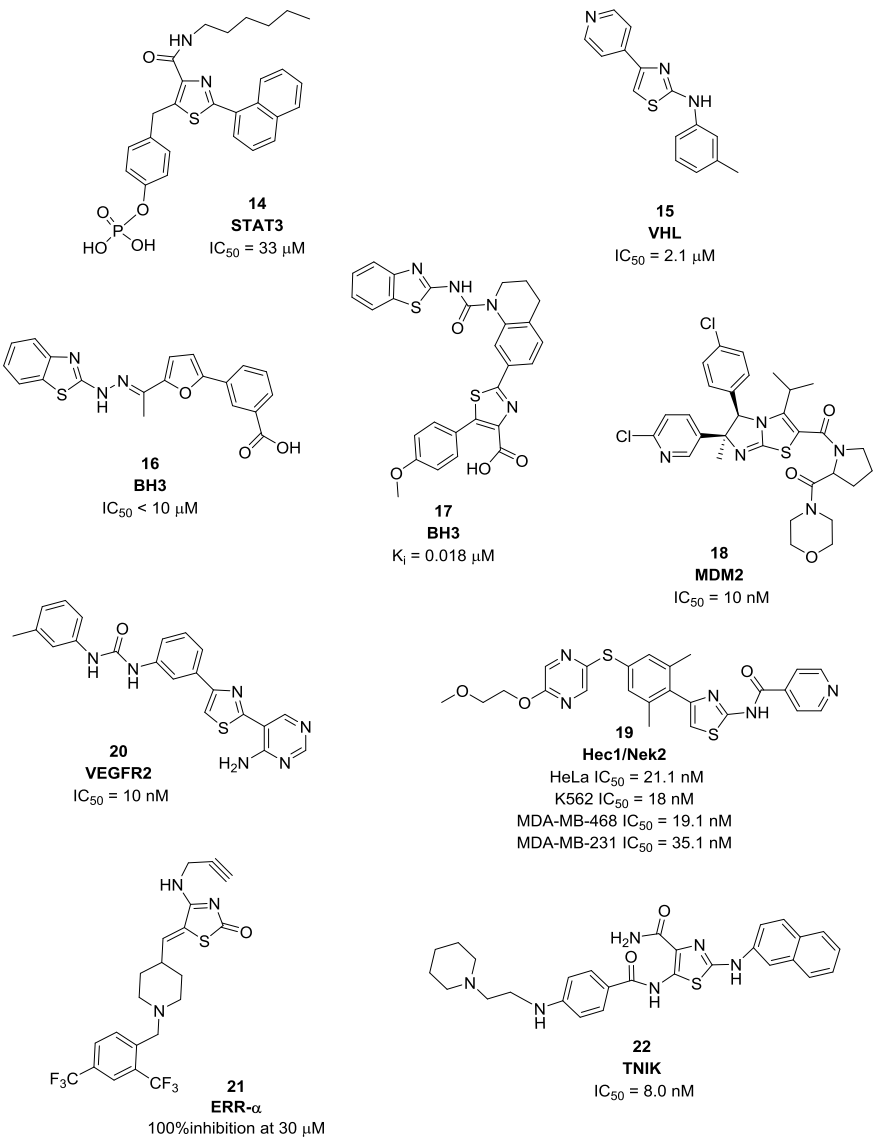


FIG. 3**. Thiazole derivatives with potential antitumor activity**

Thiazole derivatives showing miscellaneous activity

Endowed with antiviral activity

Chronic infection with hepatitis C virus (HCV) is a major health problem associated with liver cirrhosis, hepatocellular carcinoma and liver failure [132]. HCV is a member of the Flaviviridae family of RNA viruses that affect animals and humans. Interferon-α (IFN-α) and pegylated interferon (PegIFN) in combination with Ribavirin (RBV) were the choice of treatment nowadays against chronic hepatitis C until a few years ago. However, a number of patients have significant side effects, primarily related to ribavirin [133,134]. Low-molecular weight compounds that directly inhibit HCV proteins and interfere with viral replication are considered as attractive strategies to control HCV infection. In the last decade, one of the most important innovations in the treatment of HCV is Sofosbuvir, a prodrug whose main metabolite is 2'-deoxy-2'-α-fluoro-

β-C-metiluridine-5'-monophosphate, a HCV RNA polymerase inhibitor [135]. Sofosbuvir was discovered by Pharmasset and then acquired for development by Gilead Sciences. Another drug recently approved is Simeprevir (formerly TMC435; trade name Olysio) is a cyclopentane macrocyclic and thiazole derivative; an important milestone of the first-generation HCVNS3/4A protease inhibitors. It was developed by Medivir and Johnson & Johnson's pharmaceutical division Janssen Pharmaceutical [136]. In the United States, simeprevir is approved by the Food and Drug Administration for use in combination with PegIFN and RBV for hepatitis C. At last Daclatasvir (formerly BMS-790052, trade name Daklinza) is a drug for the treatment of HCV, developed by Bristol-Myers Squibb and approved in Europe on 22 August 2014. It inhibits the HCV nonstructural protein NS5A [137,138]. However, these new treatments are very expensive. The ultimate goal in the therapy of any virus infection is the elimination of the virus from the organism. As HCV is an RNA virus that does not replicate through a DNA intermediate, the prospect of a true cure seems much more realistic [139]. In 2008, Achillion Pharmaceuticals Inc. patented heteroaryl substituted thiazole compounds useful for treating hepatitis C infections. Such compounds were tested for the ability to inhibit viral replication of the hepatitis C replicon in cultured cells in which the HCV replicon construct had been incorporated. The HCV replicon system was described by Lohmann [140], and is predictive of *in vivo* anti-HCV activity. In this assay HCV replicon containing cells are treated with different concentrations of the test compound to ascertain its ability to suppress replication of the HCV replicon. Most of the tested compounds showed inhibition concentration of less than 1 μM. As an example, the structure of compound **23** is reported in **FIG 4** [141]. A few months later in 2008, also Genelabs Technologies Inc. patented substituted thiazoleamides possessing antiviral activity towards Flaviviridae family viruses such as hepatitis C virus. These compounds inhibit the enzymes involved in viral replication, including RNA dependent RNA polymerase. The percent inhibition of replication at 10 μM was determined. Several compounds displayed a percentage of inhibition higher than 95%. **FIG 4** reports the most active derivative **24**, which showed 100% inhibition [142]. The potential opportunities for the future treatment of (chronic) HCV infection are remarkably similar to the combination regimens currently used for the therapy of HIV infection. Like HCV even for HIV, drug discovery and development efforts, based on advances in the understanding of the viral life cycle, have transformed what used to be a rapid and lethal infection into a chronic condition that can be controlled for many years through combination therapies with different classes of antiviral drugs. In 2013 Gilead Sciences Inc. claimed the processes for preparing benzothiazole derivatives, (i.e. compound **25 FIG 4**) useful for treating AIDS or delaying the onset of AIDS or ARC symptoms. The compounds were tested by antiviral assays in MT4 cells infected with HIV-IIIb. The compounds show a range of EC_{50} between 1-30 μM. These results do not allow us to hypothesize any biological mechanism of action of potential new antiviral products [143].

Useful in the treatment of tuberculosis

Synthetic drugs for treating tuberculosis (TB) have been available for over half a century, but incidences of the disease continue to rise worldwide [144,145]. When coupled with the emergence of multi-drug resistant strains of *Mycobacterium tuberculosis* (MDR-TB), the scale of the problem is amplified [146-151]. In 2011 Ballell Pages *et al*., patented (pyrazol-3-yl)-1,3,4-thiadiazol-2-amine and (pyrazol-3-yl)-1,3,4-thiazol-2-amine compounds useful in the treatment of TB. Such compounds were tested with the Mycobacterium tuberculosis H37Rv Inhibition Assay (Whole Cell Assay). The measurement of the minimum inhibitory concentration (MIC) for each tested compound was performed in 96 wells flat-bottom, polystyrene microtiter plates. Isoniazid was used as a positive control. In *Mycobacterium tuberculosis*, enoyl-acyl

carrier protein reductase (ACPER), known as InhA, is an essential NADH dependent enzyme in the mycolic acid biosynthetic pathway [152] targeted by isoniazide [153]. The thiazol-2-amine derivative **26** (**Figure 4**) has been reported as a direct InhA inhibitor to have an MIC value of 0.2 µM [154]. Recently Shirude *et al*. elucidated the molecular mode of action of the derivatives with methyl thiazole scaffold and the properties that drive cellular potency [155]. They have identified for the first time a mechanism of InhA inhibition that shows a hitherto unknown neutrally charged "warhead" being accommodated in the "Y158-out" conformation at site I of the InhA. Notably, these compounds show preferential binding to the NADH-bound form of the enzyme as opposed to the NAD^+ bound form of the enzyme that may well reflect the charge complementarity between the site I group and NADH (i.e., both neutral). Additionally, the current study indicates that novel hydrophilic interactions with the protein at site III lead to favorable physicochemical properties, resulting in cellular activity.

Having detrusor muscle-contracting and urethral sphincter muscle-relaxing activity

Underactive bladder is caused by bladder contraction dysfunction, [156-160]. In 2010 Ono Pharmaceutical claimed an application for the preparation of 2-[(2-[(1R,5R)-2-oxo-5-[(1E)-7,8,8-trifluoro-4-hydroxy-4-methyl-1,7-octadien-1-yl]cyclopentyl]ethylthio]-1,3-thiazole-4-carboxylic acid (**27**, **Figure 4**) having detrusor muscle-contracting activity and urethral sphincter muscle-relaxing activity. This compound at 1 mM *in vitro* increased the contractility of rat's detrusor muscle by 180% and *in vitro* decreased the contractility of rat's urethral sphincter muscle by 83%. Therefore, the compound can be used to ameliorate bladder contraction dysfunction and/or urethral relaxation dysfunction, moreover is effective as an agent for preventing and/or treating underactive bladder, for ameliorating various symptoms associated with underactive bladder and it has little risk of side effects on the urinary system. For example, it exhibits no storage symptom, such as bladder capacity reduction offering a high risk to patients suffering with urological diseases, in an effective dose. Since the new derivative causes little changes in blood pressure or heart rate on high-dose administration as well as at an effective dose, it has little risk of side effects in patients suffering from circulatory diseases, such as hypertension. The inventor claimed that compound **27** has a good membrane permeability and superior oral absorbability; it is stable against hepatic metabolism and has a low systemic clearance. Therefore, the compound of the present invention can exert sustained drug efficacy [161].

Sodium channel inhibitors

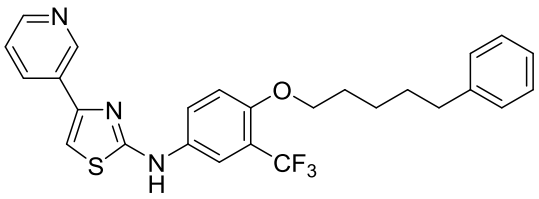
Aberrant sodium channel function is thought to underlie a variety of medical disorders [162-165] including epilepsy, arrhythmia, myotonia and pain. There are currently nine known members of the family of voltage-gated sodium channel (VGSC) [166]. In 2009 Fulp *et al*. reported the preparation of substituted N-thiazolylbenzenesulfonamides as sodium channel inhibitors. Over two hundred compounds were prepared for treating neuropathic or inflammatory. Human Embryonic Kidney (HEK) cells stably expressing the hSCN3A or hSCN9A constructs are used in a High-Throughput Screening Assays for evaluation of activity. Compound **28**, 4-bromo-2-fluoro-N-(thiazol-2-yl)-benzenesulfonamide (**FIG 4**), showed EIC_{50} of < 2 µM in HEK cells transfected with hSCN3A or hSCN9A. The compounds of the invention could be useful for treating neuropathic or inflammatory pain by the inhibition of ion flux through a voltage-gated sodium channel pain [167].

Store-operated calcium channels modulators

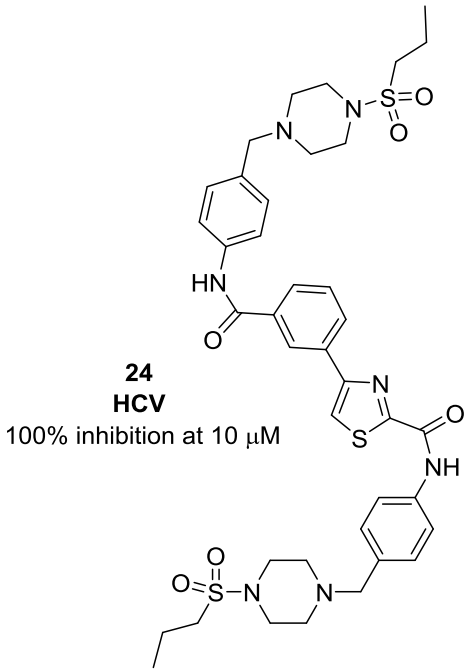
Calcium plays a vital role in cell function and survival. Store-operated calcium (SOC) influx is a process in cellular physiology that controls diverse functions such as, but not limited to, refilling of intracellular Ca^{2+} stores [168], activation of enzymatic activity [169], gene transcription [170-171] and cell proliferation. In 2012 Whitten *et al*. patented some thiazole derivatives as store-operated calcium channels modulators and their preparation and use for treatment of SOC channel-mediated diseases. Compounds described herein modulate intracellular calcium and may be used in the treatment of diseases or conditions where modulation of intracellular calcium has a beneficial effect [172,173]. Compounds described herein can inhibit store-operated calcium entry, interrupt the assembly of SOCE unit, alter the functional interactions of proteins that form store-operated calcium channel complexes, be SOC channel pore blockers, be calcium release-activated calcium channel (CRAC), a type of SOC channel, inhibit the electrophysiological current directly associated with activated SOC channels or activated CRAC channels. The diseases or disorders that may benefit from modulation of intracellular calcium include, but are not limited to, an immune system-related disease, a disease or disorder involving inflammation, cancer or other proliferative disease, kidney disease and liver disease. In one aspect, compounds described herein may be used as immunosuppressant to prevent transplant graft rejections, allogeneic or xenogeneic transplantation rejection. All the invention compounds were evaluated for their calcium channels modulatory activity; a fluorescence-based assay of store-operated calcium entry in stable Jurkat E6-1 cells was used for *in vitro* screening. In each measurement, the magnitude of the fluorescence signal, considered as a result of calcium entry into the cell, is determined by calculating the difference between the peak fluorescence signal measured after calcium addition and the initial baseline fluorescence. From this assay, it was determined that compound **29** (**FIG 4**) exhibited an IC_{50} value of <0.6µM [174].

Useful for the treatment of acute and chronic inflammatory diseases

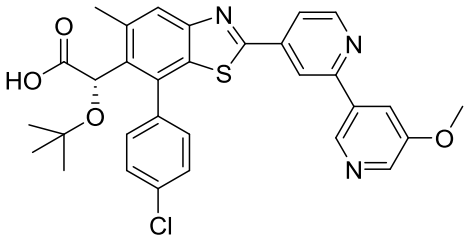
Tumor Necrosis Factor Alpha (TNF-α) is a pleiotropic cytokine involved in many other disease conditions. In 2010 Rubio Royo *et al*. patented some 5-(4-methanesulfonyl-phenyl)-thiazole derivatives for the treatment of acute and chronic inflammatory diseases. The authors of the present invention have found that thiazole derivatives have shown a number of highly interesting immune modulating effects potentially useful for the control of the pathogenic mechanisms of acute and chronic inflammatory diseases and therefore, with potential clinical applications. In particular, the compounds have been able to inhibit TNF-α production by peripheral blood mononuclear cells (PBMC) from patients suffering from a chronic inflammatory disease, such as rheumatoid arthritis, as well as to inhibit interferon gamma (IFN- α χ secretion by those cells after I-cells stimulation. Additionally, the newly compounds have been able to inhibit secretion of cytokines proinflammatory IL-8 and IL-10. The immune modulating effects were not associated to any toxic effect on mononuclear cells from peripheral blood and, moreover, these compounds did not modify the proliferative response after mitogenic stimuli. The combination, in only one small molecule, of inhibitory effects on several pro-inflammatory cytokines as INF- α, IFN- χ and IL-8 of crucial importance in the patho-physiology of systemic and organ-specific autoimmune disorders, transplantation, acute and chronic inflammatory diseases allows considering these molecules as a new category of immune modulators for targeting the cascade of pro-inflammatory cytokines. Compound **30** (**FIG 4**), prepared in a multistep synthesis that culminated in the reaction of 2-methyl-5-(4-methylsulfonylphenyl)thiazol-4-ol and cyclopentylbromide, lowered spontaneous TNF-α production by peripheral blood mononuclear cells (PBMC) at 10^{-6} M and also lowered the production of IFN-χ by PBMC from rheumatoid arthritis patients at 10^{-7} M [175].



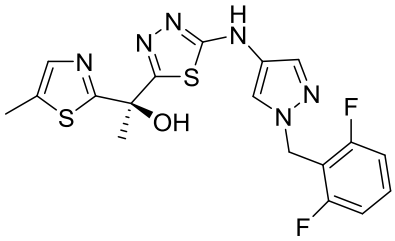
23
HCV
IC_{50} < 1.0 μM



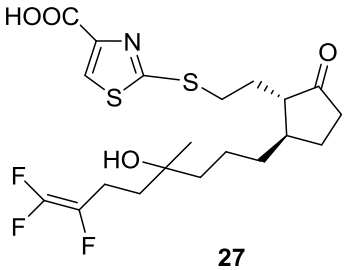
24
HCV
100% inhibition at 10 μM



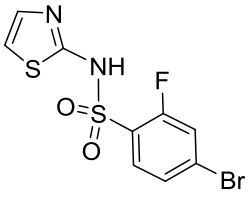
25
HIV-IIIb
EC_{50} = 1-30 μM



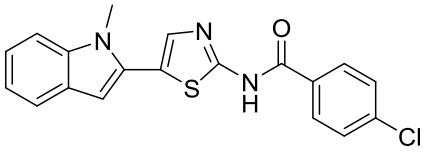
26
MTB
MIC = 0.2 μM



27
detrusor muscle activity
EC_{50} = 1.0 mM



28
VGSC
IC_{50} < 2 μM



29
SOCE
IC_{50} value of <0.6 μM

FIG 4 . Thiazole derivatives with heterogeneous activities.

Sirtuin modulators

There are seven known sirtuin members (SIRT1-SIRT7) in the histone deacetylase (HDAC) family commonly referred to as class III HDACs. Sirtuins have gained considerable attention for their impact on mammalian physiology, since they may provide novel targets for treating diseases associated with aging and perhaps extend human lifespan [176-178]. In 2008 Bennis *et al.* patented the preparation of 6-phenylimidazo [2,1-b]thiazole derivatives, i.e. derivative **31** (SRT2104) (**FIG 5**), as sirtuin modulators. In certain embodiments, sirtuin-modulating compounds may be used for a variety of therapeutic applications including, for example, increasing the lifespan of a cell, and treating and/or preventing a wide variety of diseases and disorders related to aging or stress, diabetes, obesity, neurodegenerative diseases, chemotherapeutic induced neuropathy, neuropathy associated with an ischemic event, ocular diseases and/or disorders, cardiovascular disease, etc. Sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may also be used for treating a disease or disorder in a subject that would benefit from increased mitochondrial activity, for enhancing muscle performance, for increasing muscle ATP levels, or for treating or preventing muscle tissue damage associated with hypoxia or ischemia [179]. In the patent all claims appear to be novel; however, it has to be noted that, term like "substituted" was used throughout the claims of the present application, without specification of the possible substituents. Such a non-limiting term as cited above includes chemical groups, which are structurally so remote from those of the examples that the activity cannot be predicted within the limits of qualitative structure-activity-relationship considerations. The biological profile of the new molecules is not reported in this patent, but more recently detailed studies of imidazo[2,1-*b*]thiazole derivatives have been published [180]. In particular, a summary of the properties of compound SRT2104 suggested that it could be the potential first synthetic SIRT1 activator to provide therapeutic benefit in a clinical setting and it was therefore selected for clinical development. The pharmacokinetics and tolerability of SRT2104 from two Phase I studies in man have been reported [181,182]. In animal models, SRT2104 improves glucose homeostasis and increases insulin sensitivity but in a phase II, randomized, placebo-controlled, double-blind, multi-dose study, the tolerability and pharmacokinetics of SRT2104, and its effects on glycemic control, in adults with type 2 diabetes mellitus, did not result in improved glucose or insulin control [183].

Inhibitor of T-Type Calcium Channel

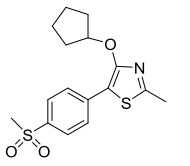
In 2008 Haln *et al.* [184] patented an invention relates to the preparation of novel guanidinothiazoles as T-type calcium channel blockers, i.e. compound **32** (**Figure 5**). This inhibitor is useful as a treating agent for disease associated with overexpression of T-type calcium channel. In the present invention, in order to search an efficient inhibitor against T-type calcium channel, a primary assay for T-type calcium channel inhibiting activity was conducted by a high-efficient assay using mammal HEK293 cell lines (originated from human kidney carcinoma cells) which specifically expresses T-type calcium channel. Through the primary assay, compounds, which show meaningful inhibition effects, were selected. The selected compounds were used in a second assay for I-type calcium channel inhibiting activity using electrophysiological whole cell patch clamp method on HEK293 cell lines. As a reference drug, mibefradil was used. In this assay compounds showed T-type calcium channel blocking activity with IC_{50} = 0.2-34.47 μM.

Treatment of Duchenne muscular dystrophy

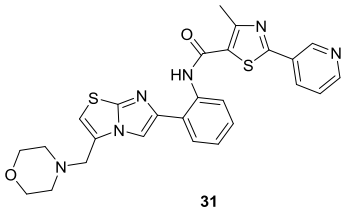
In 2009 Wynne *et al.* patented the preparation of imidazothiazoles for treatment of Duchenne muscular dystrophy (DMD), Becker muscular dystrophy and cachexia [185]. The potential activity of the compounds for use in the treatment of DMD was demonstrated in the predictive luciferase reporter assay (murine H2K cells) and in animals *in vivo* as the ability to increase the levels of utrophin protein in dystrophin deficient muscle when compared to vehicle only dosed. For example 6-(3,4-dichlorophenyl)-2-methylimidazo[2,1-b]thiazole, compound **33** (**FIG 5**), up regulated utrophin expression in murine H2K/mdx/Utro A reporter cell line cells by >401% [186].

Agonist of the TGR5 receptor

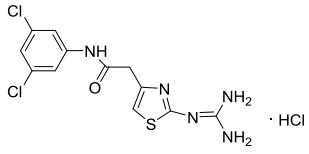
In 2013 Smith *et al*. published a patent encompassing a series of thiazolotriazole and thiazoloimidazole compounds that agonize the activity of the protein TGR5 [187]. Inventors state that these TGR5 agonists can exert their effect independently of natural bile acid ligands and may be used to regulate or activate the TGR5 signaling pathway, either *in vitro* or *in vivo*. Selected exemplary compounds of the disclosure have been tested to verify their activation activities on the 293/humanTGR5 cells measured by cAMP levels. Some of them, i.e. derivative **34** (**FIG 5**), have an IC_{50} value that is less than 0.5 μM. It should be noted that the design of TGR5 ligand with a gastrointestinal-restricted mechanism of action is being pursued by pharmaceutical companies and academic research groups as a way to overcome potential side effect linked to a systemic activation of the receptor but only few compounds have hitherto entered a clinical trial [188,189]. The biological data in this patent are few and not sufficient to exclude the agonist stimulation in other specific tissues and resulting side effects.



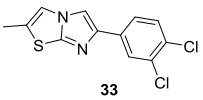
30
TNF-α by PBMC IC_{50}= 1μM
IFN-γ by PBMC IC_{50}= 0.1μM



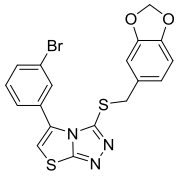
31
SIRT1
$EC_{1.5}$ (% Maximum Activation) = 0.43 μM (187%)



32
CCB
IC_{50} = 0.31±0.22 μM



33
DMD



34
TGR5
IC_{50} < 0.5 μM

FIG 5. **Thiazole derivatives with heterogeneous activities.**

TAB 1**: Lead compound claimed in the patents**

Patent	Comp*.	Title	Activity	Ref.
WO058641 (2008) Zoller G. *et al.*	**1**	Modulators of transcription for endothelial nitric oxide synthase	EC_{50} = 0.5 mM	[7]
WO068211 (2012) Kent B. J. *et al.*	**2**	Inhibitors of pro-matrix metalloproteinase activation	proMMP9 K_d = 3.6 mM proMMP13 K_d = 7 mM	[22]
US129843 (2012) Zhang Y. *et al.*	**3**	Inhibitors of pro-matrix metalloproteinase activation	proMM9 IC_{50} = 0.059 mM	[25]
US129843 (2012) Zhang Y. *et al.*	**4**	Inhibitors of pro-matrix metalloproteinase activation	proMM13 IC_{50} = 0.90 mM	[25]
US129872 (2012) Leonard KA. *et al.*	**5**	Inhibitors of pro-matrix metalloproteinase activation	proMMP9 K_d = 1.2 mM proMMP13 K_d = 1.9 mM	[26]
US302569 (2012) Jackson PF. *et al.*	**6**	Inhibitors of pro-matrix metalloproteinase activation	proMMP9 K_d = 0.10 mM proMMP13 K_d = 0.14 mM	[27]
WO137060 (2008) Allen JR. *et al.*	**7**	Inhibitors of prolyl hydroxylase activity	IC_{50} = 0.020 mM	[35]

WO066145 (2008) Kawai Y. *et al.*	**8**	Inhibitors of vascular adhesion protein 1	human VAP-1 IC_{50}=0.9 nM rat VAP-1 IC_{50} = 0.7 nM	[35]
US152287 (2011) Jordan AD. *et al.*	**9**	Inhibitors of dipeptidyl peptidase-1	IC_{50} = 0.17 mM	[43]
WO025170 (2008) Vocadlo DJ. *et al.*	**10**	Selective inhibitors of glycosidase	K_i = 5.6 mM	[49]
WO083435 (2012) Kaul R. *et al.*	**11**	Selective inhibitors of glycosidase	K_i = 0.50 mM	[52]
WO028715 (2013) Donnelly M. *et al.*	**12**	Selective inhibitors of glycosidase	IC_{50} < 0.1 mM	[55]
WO155402 (2009) Pellicciari R. *et al.*	**13**	Modulators of poly(ADP-ribose)polymerase	human PARP-1 IC_{50} = 378 nM	[59]
WO033685 (2010) Turkson J. *et al.*	**14**	Inhibitors of signal transducer and activator of transcription 3	IC_{50} = 33 µM	[67]
WO114552 (2009) Turcotte S. *et al.*	**15**	Targeting cells defective in the von Hippel-Lindau (VHL) gene	IC_{50} = 2.1 µM (renal carcinoma)	[78]
WO039553 (2009) Mckee A. J. *et al.*	**16**	BH3-only protein mimetic	IC_{50} < 10 µM	[93]
WO080478 (2010) Bayldon B. J. *et al.*	**17**	BH3-only protein mimetic	K_i = 0.018 µM	[95]

WO072655 (2008) Kawato H. *et al.*	**18**	Inhibitors of murine double minute 2	IC_{50} = 10 nM	[102]
US0190312 (2013) Huang JJ. *et al.*	**19**	Modulators of Hec1 activity	HeLa IC_{50} = 21.1 nM K562 IC_{50} = 18 nM	[111]
US20184280 (2013) Beroza PP. *et al.*	**20**	Inhibitors of vascular endothelial growth factor receptor 2	IC_{50} = 10 nM	[118]
US0072467 (2013) Matsumoto S. *et al.*	**21**	Modulators of estrogen-related receptor-α	100% inhibition at 30 µM	[126]
US0317218 (2013) Sawa M. *et al.*	**22**	Inhibitors of Traf2- and Nck-interacting kinase	IC_{50} = 8.0 nM	[127]
WO147557 (2008) Chen D. *et al.*	**23**	Endowed with antiviral activity	HCV IC_{50} < 1.0 µM	[141]
US317360 (2009) Rai R. *et al.*	**24**	Endowed with antiviral activity	HCV 100% inhibition at 10 µM	[142]
US0281434 (2013) Babaoglu K. *et al.*	**25**	Endowed with antiviral activity	HIV-IIIb EC_{50} = 1-30 µM	[143]
WO118852 (2010) Ballell P.L. *et al.*	**26**	Useful in the treatment of tuberculosis	MIC = 0.2 µM	[154]

WO143661 (2010) Ohmoto K. *et al.*	**27**	Detrusor muscle-contracting and urethral sphincter muscle-relaxing activity	EC_{50} = 1.0 mM	[161]
WO012242 (2009) Bradley F.A. *et al.*	**28**	Sodium channel inhibitors	IC_{50} < 2 µM	[167]
WO027710 (2012) Cao J. *et al.*	**29**	Store-operated calcium channels modulators	IC_{50} value of <0.6 µM	[174]
WO153226 (2009) Royo R.V. *et al.*	**30**	Treatment of acute and chronic inflammatory diseases	(TNF-α) IC_{50}= 1µM (IFN-χ) IC_{50}= 0.1µM	[175]
WO156866 (2008) Bemis J. *et al.*	**31**	Sirtuin modulators	SIRT1 $EC_{1.5}$ (% Maximum Activation) = 0.43 µM (187%)	[179]
WO018655 (2008) Hahn H.G. *et al.*	**32**	Inhibitor of T-Type Calcium Channel	IC_{50} = 0.31±0.22 µM	[184]
WO013477 (2009) Wynne G.M. *et al.*	**33**	Treatment of Duchenne muscular dystrophy	//	[174]
US0085157 Smith E. D. *et al.*	**34**	Agonist of the TGR5 receptor	TGR5 IC_{50} < 0.5 µM	[174]

*Se figure **1-5** for Structures

Conclusion

After the reading of more than 100 patents describing new thiazole derivatives it can be stated that it is still a versatile scaffold in the field of pharmaceutical research (**TAB 1**). In the interval of time, 2008-2013, considering the patents here reported, and the review regarding this azole [1,2], no new compounds have reached clinical trials. From the beginning of this century, however, several examples of thiazole derivatives are reported in the literature on clinical trials: Dasatinib, an oral tyrosine kinase inhibitor (TKI) of BCR-ABL and SRC family and Ixabepilone, an Epothilone B analog, that binds to β-tubulin and stabilizes tubulin for the therapy of metastatic solid tumor [190]; CPTH6 induces histone hypoacetylation and apoptosis in human leukemia cells [191]; Febuxostat, a nonpurine selective inhibitor of xanthine oxidase for lowering serum uric acid concentration [192]; Clomethiazole, an old analogue of thiamine and a positive allosteric modulator at the barbiturate/picrotoxin site of the GABA A receptor, still studied for its effects on the central nervous system [193]; Tetomilast (OPC-6535), a novel thiazole compound that inhibits phosphodiesterase-4 and proinflammatory functions of leukocytes including superoxide production and cytokine release, for therapy in active ulcerative colitis [194] and Simeprevir a HCVNS3/4A protease inhibitors [136]. In recent years, the design and synthesis of pharmacologically relevant heterocyclic molecules by combinatorial techniques have proven to be a promising strategy in the search for new pharmaceutical lead structures. Click chemistry is one of the powerful reactions for making carbon−heteroatom−carbon bonds in aqueous environment with a wide variety of chemical and biological applications in various fields and it is a newer approach to the synthesis of drug like molecules that can accelerate the drug discovery process by utilizing a few practical and reliable reactions. It can be underlined that all patents report in detail the multi-step reactions to synthetize the new adducts. The framework of thiazole allows various substitution patterns but generally, they are obtained by classical synthetic routes without using most modern synthetic methodologies such as click chemistry. Observing the set of final structures is evident that a lot of them contain the scaffold of the 1,3-thiazole rings variously functionalized; while a few examples of polycyclic systems are used by researchers in the design. In the latter case, the most common structures are benzothiazoles, imidazo[2,1-*b*]thiazoles, thiazolepyridine or pyrano[3,2-d]thiazole. Generally, the final products are largely well analyzed to ensure the structure and purity, as evidenced by their NMR spectra, HPLC analysis and by their mass spectra recorded. Few patents are related to the synthesis and analysis of stereoisomers, but in these cases the inventors bother to study the biological activity of the individual enantiomers or diastereoisomers after their characterization [43,55,102,161]. In some patents the screening of small libraries of thiazole derivatives have provided a low cost scaffold for further development and prompted the research to identify specific mechanisms of action, as in the case of methylthiazole derivative endowed of antitubercolar activity [155] or sirtuin modulator SRT2104 [180]. Sometimes inventors have applied structure-based design technique to discover selective inhibitors like the pyrano[3,2-d]thiazole derivatives selective inhibitors of glycosidase [49] or antitumor molecule like STF-62247 for renal carcinoma (78); sometimes also docking studies of an initial lead compound were applied like for the rational design of STAT3 inhibitors base on the peptidomimetic ISS-10 [67] or for the development of inhibitors of MDM2 structurally linked to Nutlin-3A [102]. All patents describe in detail the preliminary studies in vitro. Compounds show a range of values of activities at concentration micro or nanomolar. Some research has been further developed also with *in vivo* studies producing results for most interesting. In this regard mention may be made about *in vivo* studies of compound "alpha", a pro-MMPs inhibitor [27-30], or else *in vivo* studies with xenograft models for some antitumor thiazole derivatives like the VEGFR2 kinase inhibitors [118] or modulators of Hec1 activity [111]. The *in vivo* studies have been rarely focused

on the pharmacokinetic profile of new molecules: at this regard it can be remembered the agent for treating underactive bladder [161]. An observation part must be made for the clinical trials of the new compounds. As it is known, the clinical trials stage costs more money than any other stage of the whole process of discovering and developing a drug. As a result, pharmaceutical companies normally do not support the clinical trials process of a compound even if its efficacy *in vitro* or *in vivo* is interesting but with little market interest. Anyway for thiazole derivatives here examined, they can be mentioned clinical studies of STF-62247 [76,77] and SRT2104 [181,182] as significant achievements in specific areas. In conclusion, considering all aspects: medicinal chemistry, biological and pharmacological emerged from the analysis of patents, it is possible to evaluate the small core thiazole as a tool still valid and on which much work can be done in the future.

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