



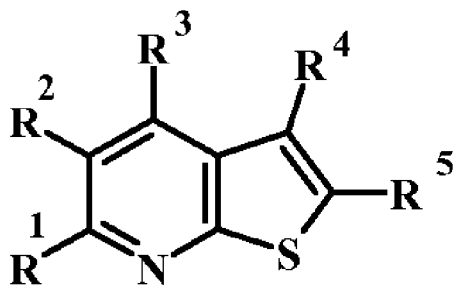
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(54) Title: THIENO[2,3-*b*]PYRIDINES AS MULTIDRUG RESISTANCE MODULATORS



(I)

(57) Abstract: The thieno[2,3-*b*]pyridines of general formula (I), wherein R¹ is Me, C₆H₅, 3,4,5-(OMe)₃C₆H₂, NH₂; R² is H, CN, COMe, COOC₁₋₄ alkyl, COOC₂H₄OMe, COOC₂H₄OPr(*n*); R³ is C₆H₄R⁶, 3,4-OCH₂O-C₆H₃; 2-furanyl; R⁶ is 4-Cl, 4-NO₂, 4-N(C₁₋₄alkyl)₂, 3-(C₁₋₄alkyloxy), 4-(C₁₋₄alkyloxy), 3,4-(C₁₋₄alkyloxy)₂, 3,4,5-(C₁₋₄alkyloxy)₃; R⁴ is NH₂, NHCOMe; R⁵ is CN, COMe, COC₆H₄R⁷; CO-(2-naphthyl); R⁷ is H, 4-F, 4-Cl, 3-OMe, 4-OMe, 2,4-(OMe)₂, 3,4,5-(OMe)₃, as multidrug resistance modulators to increase the effectiveness of chemotherapy in cancer treatment.

THIENO[2,3-*b*]PYRIDINES AS MULTIDRUG RESISTANCE MODULATORS

Field of the invention

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This invention relates to novel thieno[2,3-*b*]pyridine derivatives and their use as multidrug resistance modulators to increase the effectiveness of chemotherapy in cancer treatment.

10 Background of the invention

Chemotherapy as cancer therapy has found clinical application in the treatment of almost every type of cancer. One of the major problems in cancer chemotherapy is the development of resistance to cytotoxic drugs. Patients who do not respond to a first course of chemotherapy relapse because tumor cells develop resistance against chemotherapeutic agents or has acquired resistance to cytotoxic agents used in a previous treatment. A tumor may also manifest resistance to a cytotoxic agent to which it has not been previously exposed. Multidrug resistance (MDR) in tumor cells has a significant impact on the efficacy of cancer chemotherapy and appears as a major obstacle in the modern cancer treatment.

15 MDR is mainly related to the expression of the adenosine triphosphate ATP-binding cassette (ABC) transporters. P-glycoprotein (P-gp) (the best studied target for reverting MDR), multidrug resistance-associated protein (MRP1) and the breast cancer resistance protein (BCRP) as a major MDR proteins actively transport a wide variety of structurally different substrates out of the tumor cells, thereby decreasing their intracellular concentrations. Many

20 actual chemotherapeutic agents are considered as potential P-gp, MRP1 and BCRP substrates (Szakács et al, Nat Rev Drug Discov 3: 219-34 (2006); Szakács et al., Drug Resistance in Cancer Cells 1-20 (2009)).

From all numerous efforts to overcome MDR like transcription control of P-gp expression the most promising approach has been the development of MDR modulators that are able to increase the intracellular drug levels in co-application with MDR substrates by the

30 efflux pump inhibition. Substances of different groups have been used as P-gp inhibitors. Ca²⁺ channel blocker verapamil is the most investigated and often used as reference

compound, but unfortunately, in combination with actual anticancer drugs cardiotoxicity was observed (Pennock et al., J Natl Cancer Inst 83: 105-10 (1991)).

The functional unit of an ABC transporter contains two transmembrane domains (TMDs) and two nucleotide ATP-binding domains (NBDs). Transporters such as ABCG2 (BCRP) contain only one TMD and one NBD forms dimers.

Several ABC transporters have been found to be overexpressed in cancer cell lines under selective conditions. It was shown that the major mechanism of MDR in most cultured cancer cells involves P-gp, MRP1 and BCRP transport proteins. ABC transporters control not only the drug release to the cell, but also the intracellular compartmentalization or division between the cytoplasm and nucleus.

P-gp, a member of the ABCB subfamily, confers the strongest resistance to the wide variety of compounds. P-gp transports vinca alkaloids, anthracyclines, epipodophyllotoxins and taxanes. P-gp is normally expressed in epithelium of the liver, kidney and gastrointestinal tract at pharmacological barrier sites in stem cells and cells of immune system.

MRP1 is a member of ABCC subfamily and confers resistance to several hydrophobic compounds that are also P-gp substrates. However, MRP1 can export glutathione, glucuronate or sulphate conjugates of organic anions. MRP1 is expressed in wide range of tissues, tumors and cancer cell lines.

BCRP is a member of ABCG subfamily. The substrate specificity of BCRP overlaps considerably with that of P-gp. BCRP is involved in the mechanism of resistance to a topoisomerase I inhibitor (topotecan) or topoisomerase II inhibitor (mitoxantrone). BCRP does not act on paclitaxel or vincristine transport, which are excreted by P-gp, and BCRP is involved in excretion of a camptothecin derivative, which is barely transported by P-gp (Kruijtzter et al., J Clin Oncol 20: 2943-50 (2002)). BCRP is expressed in many normal tissues, including liver, placenta, brain, hematopoietic stem cells and other types of stem cells.

Besides the clinically important drugs, several fluorescent compounds are transported by P-gp, MRP1 and BCRP such as rhodamine 123 (P-gp), calcein (MRP1), Hoechst 33342 (P-gp, BCRP). These fluorescent compounds are used in studies of ABC transporters in cell lines.

Therefore, there is an actual need in MDR modulators, which are non-toxic, have weak influence (or no influence) on cardiovascular system and would effectively inhibit

adenosine triphosphate binding cassette transport - modulate multidrug resistance in tumor cells and rise effectiveness of chemotherapy.

At the same time, thieno[2,3-*b*]pyridines are known to be biologically active substances, possessing, for example, PI3K inhibition activity, antiviral activity, osteogenesis promotion activity and modulating properties towards metabotropic glutamate receptors.

Thus, thieno[2,3-*b*]pyridines having phosphatidylinositide 3-kinases (PI3K) inhibitors activity have been disclosed in WO 2012/003262 A1.

Thieno[2,3-*b*]pyridines exhibiting nicotinamide adenine dinucleotide phosphate (NADPH) oxidase II inhibitor activity have been disclosed in WO 2011/075559 A1.

Thieno[2,3-*b*]pyridines proposed as inhibitors of human mitogen-activated protein kinase (MEK) enzymes are disclosed in WO 2009/153554 A1, WO 2009/013462 A1, WO 2009/093008 A1, WO 2007/088345 A1.

Thieno[2,3-*b*]pyridines possess antiviral activity, and more specifically are useful for treating HIV (Human Immunodeficiency Virus) infection (WO 2010/130842 A1, WO 2009/062288 A1) or Hepatitis C (US 2006/0019976 A1).

Thieno[2,3-*b*]pyridines have been reported to be useful for promoting osteogenesis, suppressing bone resorption and/or improving bone density; for prevention or treatment of osteopathy (for example, osteoporosis, in particular postmenopausal osteoporosis, senile osteoporosis or secondary osteoporosis caused by the use of steroids or immunosuppressants), osteopenia or bone destruction associated with rheumatoid arthritis, Paget's disease of bone, bone fracture or dysostosis due to dwarfism or osteoarthritis (EP 1764367 A1).

Thieno[2,3-*b*]pyridines have been claimed as potent modulators of metabotropic glutamate receptors (for mGluR5 and mGluR1 receptor subtype reported in WO 2007/072090 A1 and WO 2007/072091 A1, mGluR2 receptor subtype reported in WO 2006/030031 A1).

Thieno[2,3-*b*]pyridines have I κ B kinase (IKK) complex inhibitor activity, therefore are useful in the treatment of IKK mediated diseases including autoimmune diseases, inflammatory diseases, cardiovascular disease and cancer have been disclosed in US 2007/0293533 A1.

Thieno[2,3-*b*]pyridines as inhibitors of tumor necrosis factor (TNF) have been disclosed in WO 2006/074919 A2.

Thieno[2,3-*b*]pyridines have been reported as vanilloid receptor 1 (VR1 or TRPV1) antagonists in WO 2006/068618 A1.

Thieno[2,3-*b*]pyridines are active on the GABA_B receptor and can be used in treating CNS disorders (WO 2006/063732 A1).

Thieno[2,3-*b*]pyridines which modulate K⁺ channel (possess inhibitor activity) have been described in WO 2006/061642 A1.

5 Thieno[2,3-*b*]pyridines that are selective allosteric modulators of the M₄ subtype of muscarinic receptors, useful for treatment of disorders associated with M₄ muscarinic receptors have been reported in WO 2006/047124 A1.

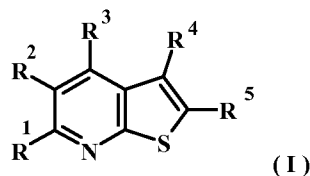
However, there are no reports of thieno[2,3-*b*]pyridines exhibiting multidrug resistance modulating properties.

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Description of the Invention

We have unexpectedly discovered, that thieno[2,3-*b*]pyridines of general formula (I) are effective as MDR modulators:

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wherein

R¹ is C₁₋₄alkyl, C₆H₅, 3,4,5-(OMe)₃C₆H₂, NH₂;

R² is H, CN, COMe, COOC₁₋₄alkyl, COOC₂H₄OC₁₋₄alkyl;

20 R³ is C₆H₄R⁶, 3,4-OCH₂O-C₆H₃; 2-furanyl;

R⁶ is 4-Cl, 4-NO₂, 4-N(C₁₋₄alkyl)₂, 3-(C₁₋₄alkyloxy), 4-(C₁₋₄alkyloxy),

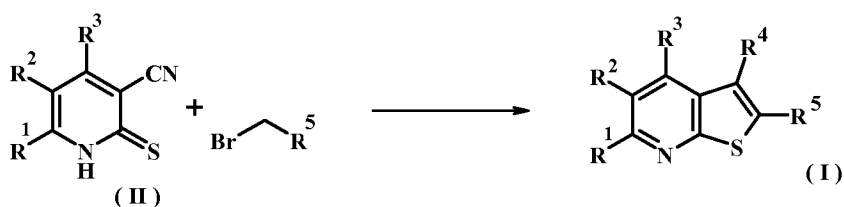
3,4-(C₁₋₄alkyloxy)₂, 3,4,5-(C₁₋₄alkyloxy)₃;

R⁴ is NH₂, NHCOMe;

R⁵ is CN, COMe, COC₆H₄R⁷; CO-(2-naphthyl);

25 R⁷ is H, 4-F, 4-Cl, 3-OMe, 4-OMe, 2,4-(OMe)₂, 3,4,5-(OMe)₃

Thieno[2,3-*b*]pyridines of the formula (I) were synthesized by the following scheme.



By treatment of 5-cyano-1,6-dihydro-6-thioxopyridines with carbo function containing alkylhalides in presence of base first alkylation takes place. The formed intermediates 2-alkylsulphanyl-3-cyanopyridines undergo Thorpe-Ziegler cyclization giving thieno[2,3-*b*]pyridines (compounds of formula (I)). Amino group in position 3 ($R^4 = NH_2$) was acetylated to give compounds of the formula (I) ($R^4 = NHCOMe$). A series of original 5-cyano-1,6-dihydro-6-thioxopyridines and α,β -unsaturated ketones as intermediates were synthesized.

Thieno[2,3-*b*]pyridines of the formula (I) were tested to reveal MDR-blocking activity. The potency of P-glycoprotein-mediated MDR modulator *in vitro* was evaluated in the drug sensitive human sarcoma MES-SA cells and doxorubicin (DOX) resistant cells by accumulation of fluorescent substrate - rhodamine 123. Effects on MRP1-mediated drug efflux were observed in the DOX resistant human lung carcinoma H69AR cells using calcein AM assay. BCRP1-mediated drug efflux was also evaluated using fluorescent substrate Hoechst 33342 in the mitoxantrone resistant human sarcoma MESSA/MX2 cells. The half-maximal effective concentrations (EC_{50}) were calculated from dose response curve as the most effective method to compare MDR-modulating activities of compounds of formula (I) with a reference compounds Verapamil, MK-571 and Glivec.

As Ca^{2+} channel blocker Verapamil in combination with actual anticancer drugs have revealed cardio toxicity, influence of compounds of the formula (I) on cardiovascular system as well as their toxicity were tested. Changes in intracellular $[Ca^{2+}]$ concentration were studied using A7R5 (rat aorta smooth muscle) cells and Fluo-4 NW Calcium Assay Kit ("Invitrogen", Sweden) according to manufacturer's instructions.

MDR modulating activity, intracellular Ca^{2+} values and LD_{50} values of tested compounds of the formula (I) are given in table 3.

2-COAr, 3-NH₂, 4-Ar, 5-COMe or 5-COOAlk and 6-Me groups are essential and together with thieno[2,3-*b*]pyridine scaffold determine MDR modulating activity. Exchange of COAr group in position 2 for COMe, CONHAr or CN groups, acylation of amino group in position 3, removing of acetyl or ester groups in position 5 (in case of 5-H and 5-CN substituents), as well as exchange of Me group in position 6 with aryl or amino groups lead to diminution of activity. The series bearing $R^1 = Me$, $R^2 = COOAlk$, $R^3 = 3,4,5-(OMe)_3C_6H_2$ and $R^4 = 4-MeOC_6H_4CO$ are the most potent MDR modulators exceeding significantly reference compounds Verapamil, MK-571 and Glivec. Activity is increased by changing of COMe group for COOMe, reaching maximum in case of COOEt and COOC₂H₄OMe groups,

but slightly reduced by introduction of more lipophylic COOC₂H₄OC₃H₇ and COOBu(n) groups in position 5.

Substitution of R³ = 3,4,5-(OMe)₃C₆H₂ group in position 4 for 3,4-(OMe)₂C₆H₃, 3-OMeC₆H₄, 4-OMeC₆H₄, 4-OMeC₆H₄, 4-OHC₆H₄, 4-ClC₆H₄ groups, as well as substitution of R⁴ = 4-OMeC₆H₄CO group in position 2 for 2,4-(OMe)₂C₆H₃CO, 3-OMeC₆H₄CO, C₆H₅CO, 4-ClC₆H₄CO and 4-FC₆H₄CO groups lead to weakening of activity.

Thieno[2,3-*b*]pyridines bearing R¹ = Me, R² = COOAlk, R³ = 3,4,5-(OMe)₃C₆H₂ and R⁴ = COAr (compounds OSI-9767, OSI-9995, OSI-10102, OSI-10103, OSI-10205, OSI-10206, OSI-10267) inhibit three MDR transport proteins: P-gp, MRP1 and BCRP1.

Thieno[2,3-*b*]pyridines bearing R¹ = Me, R² = COOAlk, R³ = 4-NMe₂C₆H₄ or (OMe)_nC₆H_{5-n} and R⁴ = OMeC₆H₄CO groups (compounds OSI-10175, OSI-10214, OSI-10215, OSI-10240, OSI-10266, OSI-10276) selectively inhibit P-gp and BCRP1.

Thieno[2,3-*b*]pyridines with varied MDR-blocking activity (OSI-9767, OSI-9995, OSI-9998, OSI-9999, OSI-10102, OSI-10103, OSI-10125, OSI-10205, OSI-10206, OSI-10267, OSI-10302) exceeding significantly reference compounds Verapamil, MK-571, Reversan and Glivec, reveal more than ten times lower Ca²⁺ antagonist effect than Verapamil (less cardiovascular side effects expected) and being non-toxic (LD₅₀ > 2000 mg/kg) are potential agents to overcome MDR problem in clinic.

According to the current invention, the most preferred thieno[2,3-*b*]pyridines (I) are those listed in the Table 1.

Table 1. Most preferred thieno[2,3-*b*]pyridines (I).

Compound No.	Compound name	
1	OSI-10036	5-Acetyl-3-amino-4-(3,4-dimethoxyphenyl)-2-(4-methoxybenzoyl)-6-methyl-thieno[2,3- <i>b</i>]pyridine
2	OSI-10039	5-Acetyl-3-amino-2-(2,4-dimethoxybenzoyl)-4-(3,4-dimethoxyphenyl)-6-methyl-thieno[2,3- <i>b</i>]pyridine
3	OSI-10125	5-Acetyl-3-amino-2-(4-methoxybenzoyl)-6-methyl-4-(3,4,5-trimethoxyphenyl)-thieno[2,3- <i>b</i>]pyridine
4	OSI-10120	5-Acetyl-3-amino-2-(2,4-dimethoxybenzoyl)-6-methyl-4-(3,4,5-trimethoxyphenyl)-thieno[2,3- <i>b</i>]pyridine
5	OSI-10215	3-Amino-2-(4-methoxybenzoyl)-4-(4-methoxyphenyl)-6-methyl-thieno[2,3- <i>b</i>]pyridine-5-carboxylic acid methyl ester
6	OSI-9998	3-Amino-4-(3,4-dimethoxyphenyl)-2-(4-methoxybenzoyl)-6-methyl-thieno[2,3- <i>b</i>]pyridine-5-carboxylic acid methyl ester
7	OSI-10106	3-Amino-2-(2,4-dimethoxybenzoyl)-4-(3,4-dimethoxyphenyl)-6-methyl-thieno[2,3- <i>b</i>]pyridine-5-carboxylic acid methyl ester
8	OSI-10105	3-Amino-2-(4-fluorobenzoyl)-6-methyl-4-(3,4,5-trimethoxyphenyl)thieno[2,3- <i>b</i>]pyridine-5-carboxylic acid methyl ester
9	OSI-10104	3-Amino-2-(4-chlorobenzoyl)-6-methyl-4-(3,4,5-trimethoxyphenyl)thieno[2,3- <i>b</i>]pyridine-5-carboxylic acid methyl ester

10	OSI-10103	3-Amino-2-(4-methoxybenzoyl)-6-methyl-4-(3,4,5-trimethoxyphenyl)thieno[2,3- <i>b</i>]pyridine-5-carboxylic acid methyl ester
11	OSI-10102	3-Amino-2-(2,4-dimethoxybenzoyl)-6-methyl-4-(3,4,5-trimethoxyphenyl)thieno[2,3- <i>b</i>]pyridine-5-carboxylic acid methyl ester
12	OSI-10185	3-Amino-6-methyl-2-(3,4,5-trimethoxybenzoyl)-4-(3,4,5-trimethoxyphenyl)-thieno[2,3- <i>b</i>]pyridine-5-carboxylic acid methyl ester
13	OSI-10164	3-Amino-4-phenyl-2-(4-methoxybenzoyl)-6-methylthieno[2,3- <i>b</i>]pyridine-5-carboxylic acid ethyl ester
14	OSI-10301	3-Amino-4-(4-hydroxyphenyl)-2-(4-methoxybenzoyl)-6-methylthieno[2,3- <i>b</i>]pyridine-5-carboxylic acid ethyl ester
15	OSI-10237	3-Amino-2-(4-methoxybenzoyl)-4-(3-methoxyphenyl)-6-methylthieno[2,3- <i>b</i>]pyridine-5-carboxylic acid ethyl ester
16	OSI-10302	3-Amino-4-(4-hydroxy-3-methoxyphenyl)-2-(4-methoxybenzoyl)-6-methylthieno[2,3- <i>b</i>]pyridine-5-carboxylic acid ethyl ester
17	OSI-10214	3-Amino-2-(4-methoxybenzoyl)-4-(4-methoxyphenyl)-6-methylthieno[2,3- <i>b</i>]pyridine-5-carboxylic acid ethyl ester
18	OSI-9992	3-Amino-4-(3,4-dimethoxyphenyl)-2-(4-fluorobenzoyl)-6-methylthieno[2,3- <i>b</i>]pyridine-5-carboxylic acid ethyl ester
19	OSI-9994	3-Amino-2-(4-chlorobenzoyl)-4-(3,4-dimethoxyphenyl)-6-methylthieno[2,3- <i>b</i>]pyridine-5-carboxylic acid ethyl ester
20	OSI-9993	3-Amino-4-(3,4-dimethoxyphenyl)-2-(4-methoxybenzoyl)-6-methylthieno[2,3- <i>b</i>]pyridine-5-carboxylic acid ethyl ester
21	OSI-9991	3-Amino-2-(2,4-dimethoxybenzoyl)-4-(3,4-dimethoxyphenyl)-6-methylthieno[2,3- <i>b</i>]pyridine-5-carboxylic acid ethyl ester
22	OSI-9999	3-Amino-2-(4-fluorobenzoyl)-4-(3,4,5-trimethoxyphenyl)-6-methylthieno[2,3- <i>b</i>]pyridine-5-carboxylic acid ethyl ester
23	OSI-10034	3-Amino-2-(4-chlorobenzoyl)-4-(3,4,5-trimethoxyphenyl)-6-methylthieno[2,3- <i>b</i>]pyridine-5-carboxylic acid ethyl ester
24	OSI-9995	3-Amino-2-(4-methoxybenzoyl)-6-methyl-4-(3,4,5-trimethoxyphenyl)thieno[2,3- <i>b</i>]pyridine-5-carboxylic acid ethyl ester
25	OSI-9767	3-Amino-2-(2,4-dimethoxybenzoyl)-6-methyl-4-(3,4,5-trimethoxyphenyl)-thieno[2,3- <i>b</i>]pyridine-5-carboxylic acid ethyl ester
26	OSI-10266	3-Amino-4-(4-ethoxyphenyl)-2-(4-methoxybenzoyl)-6-methylthieno[2,3- <i>b</i>]pyridine-5-carboxylic acid ethyl ester
27	OSI-10257	3-Amino-4-(4-butoxyphenyl)-2-(4-methoxybenzoyl)-6-methylthieno[2,3- <i>b</i>]pyridine-5-carboxylic acid ethylester
28	OSI-10241	3-Amino-4-(4-chlorophenyl)-2-(4-methoxybenzoyl)-6-methylthieno[2,3- <i>b</i>]pyridine-5-carboxylic acid ethylester
29	OSI-10240	3-Amino-4-(4-dimethylaminophenyl)-2-(4-methoxybenzoyl)-6-methylthieno[2,3- <i>b</i>]pyridine-5-carboxylic acid ethylester
30	OSI-10243	3-Amino-2-(4-methoxybenzoyl)-6-methyl-4-(3,4,5-trimethoxyphenyl)thieno[2,3- <i>b</i>]pyridine-5-carboxylic acid butyl ester
31	OSI-10276	3-Amino-2-(4-methoxybenzoyl)-4-(4-methoxyphenyl)-6-methylthieno[2,3- <i>b</i>]pyridine-5-carboxylic acid 2-methoxyethyl ester
32	OSI-10267	3-Amino-2-benzoyl-6-methyl-4-(3,4,5-trimethoxyphenyl)thieno[2,3- <i>b</i>]pyridine-5-carboxylic acid 2-methoxyethyl ester
33	OSI-10175	3-Amino-2-(3-methoxybenzoyl)-6-methyl-4-(3,4,5-trimethoxyphenyl)thieno[2,3- <i>b</i>]pyridine-5-carboxylic acid 2-methoxyethyl ester
34	OSI-10205	3-Amino-2-(4-methoxybenzoyl)-6-methyl-4-(3,4,5-trimethoxyphenyl)thieno[2,3- <i>b</i>]pyridine-5-carboxylic acid 2-methoxyethyl ester
35	OSI-10206	3-Amino-2-(2,4-dimethoxybenzoyl)-6-methyl-4-(3,4,5-trimethoxyphenyl)-thieno[2,3- <i>b</i>]pyridine-5-carboxylic acid 2-methoxyethyl ester
36	OSI-10268	3-Amino-6-methyl-2-(3,4,5-trimethoxybenzoyl)-4-(3,4,5-trimethoxyphenyl)-thieno[2,3- <i>b</i>]pyridine-5-carboxylic acid 2-methoxyethyl ester
37	OSI-10173	3-Amino-2-(2,4-dimethoxybenzoyl)-6-methyl-4-(3,4,5-trimethoxyphenyl)-thieno[2,3- <i>b</i>]pyridine-5-carboxylic acid 2-propoxyethyl ester
38	OSI-10269	3-Amino-6-methyl-2-(3,4,5-trimethoxybenzoyl)-4-(3,4,5-trimethoxyphenyl)-thieno[2,3- <i>b</i>]pyridine-5-carboxylic acid 2-propoxyethylester
39	OSI-10248	3-Amino-4,6-bis-(3,4,5-trimethoxyphenyl)-2-(4-methoxybenzoyl)thieno[2,3- <i>b</i>]-

		pyridine-5-carboxylic acid ethyl ester
40	OSI-10281	3-Amino-4,6-bis-(3,4,5-trimethoxyphenyl)-2-(3,4,5-trimethoxybenzoyl)-thieno[2,3- <i>b</i>]pyridine-5-carboxylic acid ethyl ester
41	OSI-10249	3-Amino-6-methyl-2-(naphthalene-2-carbonyl)-4-(3,4,5-trimethoxyphenyl)-thieno[2,3- <i>b</i>]pyridine-5-carboxylic acid methyl ester
42	OSI-10033	3-Amino-4-(benzo[1,3]dioxol-5-yl)-2-(4-methoxybenzoyl)-6-methylthieno[2,3- <i>b</i>]pyridine-5-carboxylic acid ethyl ester
43	OSI-10279	3-Amino-4-(furan-2-yl)-2-(4-methoxybenzoyl)-6-methylthieno[2,3- <i>b</i>]pyridine-5-carboxylic acid ethyl ester
44	OSI-10280	3,6-Diamino-2-(4-methoxybenzoyl)-4-(3,4,5-trimethoxyphenyl)thieno[2,3- <i>b</i>]pyridine-5-carbonitrile
45	OSI-10000	3-Amino-4,6-dimethyl-2-(2,4-dimethoxybenzoyl)thieno[2,3- <i>b</i>]pyridine
46	OSI-10291	3-Amino-2-(4-methoxybenzoyl)-6-methyl-4-(3,4,5-trimethoxyphenyl)thieno[2,3- <i>b</i>]pyridine
47	OSI-10183	3-Amino-4,6-bis-(3,4,5-trimethoxyphenyl)-2-(4-chlorobenzoyl)thieno[2,3- <i>b</i>]pyridine
48	OSI-10184	3-Amino-4,6-bis-(3,4,5-trimethoxyphenyl)-2-(4-methoxybenzoyl)thieno[2,3- <i>b</i>]pyridine
49	OSI-10272	3-Acetylamino-2-(4-methoxybenzoyl)-6-methyl-4-(3,4,5-trimethoxyphenyl)-thieno[2,3- <i>b</i>]pyridine-5-carboxylic acid 2-methoxyethyl ester

According to the current invention, the most preferred compounds of formula (II) (intermediates in the synthesis of compounds of formula (I)) are listed in the Table II.

- 5 Table 2. Most preferred compounds of formula (II) - intermediates in the synthesis of compounds of formula (I).

Compound No.	
50	5-Acetyl-4-(3,4-dimethoxyphenyl)-6-methyl-2-thioxo-1,2-dihydropyridine-3-carbonitrile
51	5-Acetyl-6-methyl-2-thioxo-4-(3,4,5-trimethoxyphenyl)-1,2-dihydropyridine-3-carbonitrile
52	5-Cyano-4-(3,4-dimethoxyphenyl)-2-methyl-6-thioxo-1,6-dihydropyridine-3-carboxylic acid methyl ester
53	5-Cyano-2-methyl-6-thioxo-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyridine-3-carboxylic acid methyl ester
54	5-Cyano-4-(3-methoxyphenyl)-2-methyl-6-thioxo-1,6-dihydropyridine-3-carboxylic acid ethyl ester
55	5-Cyano-4-(4-hydroxy-3-methoxyphenyl)-2-methyl-6-thioxo-1,6-dihydropyridine-3-carboxylic acid ethyl ester
56	5-Cyano-4-(4-ethoxyphenyl)-2-methyl-6-thioxo-1,6-dihydropyridine-3-carboxylic acid ethyl ester
57	4-(4-Butoxyphenyl)-5-cyano-2-methyl-6-thioxo-1,6-dihydropyridine-3-carboxylic acid ethyl ester
58	5-Cyano-2-methyl-6-thioxo-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyridine-3-carboxylic acid <i>n</i> -butyl ester
59	5-Cyano-2-methyl-6-thioxo-4-(4-methoxyphenyl)-1,6-dihydropyridine-3-carboxylic acid 2-methoxyethyl ester
60	5-Cyano-2-methyl-6-thioxo-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyridine-3-carboxylic acid 2-methoxyethyl ester
61	5-Cyano-2-methyl-6-thioxo-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyridine-3-carboxylic acid 2-propoxyethyl ester
62	5-Cyano-2,4-bis-(3,4,5-trimethoxyphenyl)-6-thioxo-1,6-dihydropyridine-3-carboxylic acid ethyl ester

63	6-Amino-2-thioxo-4-(3,4,5-trimethoxyphenyl)-1,2-dihydropyridine-3,5-dicarbonitrile
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MDR modulating activity of the preferred thieno[2,3-*b*]pyridines of formula (I) are summarized in the Table 3.

5 Table 3. MDR modulating activity of the preferred thieno[2,3-*b*]pyridines of formula (I).

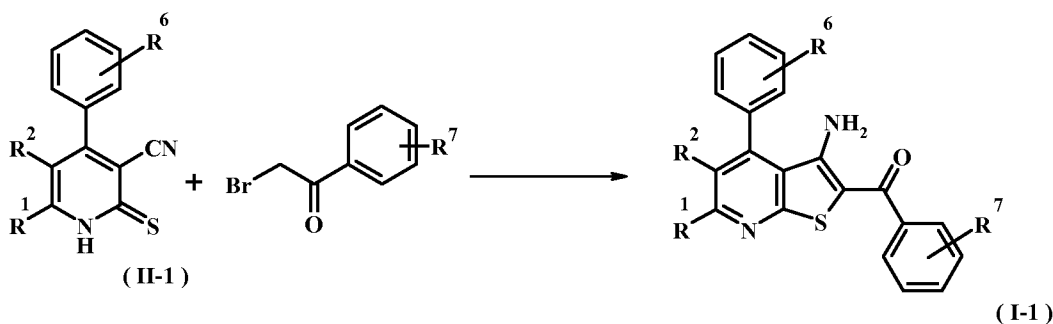
Compound No.	Compound name	MDR, EC ₅₀ , μM			Ca ²⁺ A7R5, IC ₅₀ , μM	LD ₅₀ , mg/kg
		P-gp	MRP1	BCRP1		
	Verapamil	7.1 ± 2.0	27.8 ± 0.8	37.3 ± 7.0	0.3 ± 0.1	962
	MK-571	No effect	12.4 ± 2.2	-	No effect	752
	Glivec	No effect	-	9.6 ± 1.2	>100	885
1	OSI-10036	11.5 ± 0.5	8.9 ± 1.4	9.1 ± 0.1	24.0 ± 1.2	> 2000
2	OSI-10039	13.8 ± 0.3	11.8 ± 1.2	5.5 ± 0.4	15.0 ± 1.1	> 2000
3	OSI-10125	3.8 ± 0.1	6.6 ± 1.0	2.6 ± 0.6	14.0 ± 0.9	> 2000
4	OSI-10120	8.5 ± 0.7	7.7 ± 0.4	No effect	No effect	1073
5	OSI-10215	4.5 ± 0.2	No effect	0.7 ± 0.1	15.4 ± 2.0	> 2000
6	OSI-9998	5.6 ± 0.2	11.9 ± 1.3	3.6 ± 0.6	6.0 ± 0.8	2808
7	OSI-10106	10.3 ± 1.5	41.4 ± 1.6	4.1 ± 0.9	5.6 ± 1.4	1045
8	OSI-10105	11.0 ± 1.0	10.4 ± 1.2	2.7 ± 0.5	3.0 ± 0.8	> 2000
9	OSI-10104	10.8 ± 1.6	No effect	1.4 ± 0.3	3.0 ± 0.2	1423
10	OSI-10103	1.5 ± 0.2	4.5 ± 0.5	4.0 ± 0.8	9.0 ± 0.7	> 2000
11	OSI-10102	6.9 ± 0.9	7.1 ± 0.6	1.7 ± 0.2	18 ± 1.0	> 2000
12	OSI-10185	No effect	No effect	No effect	-	-
13	OSI-10164	No effect	No effect	No effect	-	-
14	OSI-10301	26.0 ± 4.2	30.7 ± 6.0	10.2 ± 1.6	4.3 ± 0.7	231
15	OSI-10237	No effect	No effect	No effect	-	-
16	OSI-10302	5.3 ± 0.8	4.9 ± 0.2	3.3 ± 1.1	3.7 ± 0.4	1872
17	OSI-10214	6.5 ± 0.9	No effect	0.4 ± 0.1	100.0 ± 11.0	> 2000
18	OSI-9992	26.7 ± 0.9	22.1 ± 2.0	3.0 ± 0.8	7.0 ± 1.2	> 2000
19	OSI-9994	28.4 ± 2.5	No effect	5.7 ± 0.9	11.0 ± 0.7	> 2000
20	OSI-9993	9.9 ± 1.6	9.4 ± 1.6	1.1 ± 0.3	3.0 ± 0.4	2037
21	OSI-9991	8.2 ± 1.0	8.0 ± 0.2	2.0 ± 0.4	1.2 ± 0.2	2361
22	OSI-9999	4.0 ± 0.2	8.6 ± 0.1	1.1 ± 0.4	20.0 ± 2.0	2938
23	OSI-10034	3.2 ± 0.7	30.4 ± 1.3	8.1 ± 1.3	4.0 ± 0.4	2705
24	OSI-9995	0.3 ± 0.1	5.2 ± 0.6	2.5 ± 0.5	19 ± 3.0	> 2000
25	OSI-9767	6.4 ± 0.6	12.4 ± 0.4	2.6 ± 0.3	46 ± 1.4	> 2000
26	OSI-10266	4.2 ± 0.7	No effect	1.3 ± 0.2	> 100	> 2000
27	OSI-10257	No effect	No effect	No effect	-	-
28	OSI-10241	10.0 ± 0.2	No effect	2.7 ± 0.6	> 100	> 2000
29	OSI-10240	1.8 ± 0.6	No effect	0.5 ± 0.1	35.0 ± 3.0	> 2000
30	OSI-10243	1.5 ± 0.1	No effect	0.4 ± 0.08	5.0 ± 0.7	> 2000
31	OSI-10276	1.0 ± 0.1	No effect	0.8 ± 0.1	21.0 ± 4.0	> 2000
32	OSI-10267	1.4 ± 0.1	3.9 ± 0.6	1.3 ± 0.2	2.2 ± 0.3	> 2000
33	OSI-10175	1.3 ± 0.1	No effect	1.2 ± 0.3	6.2 ± 0.7	> 2000
34	OSI-10205	0.3 ± 0.2	1.1 ± 0.1	0.2 ± 0.05	3.1 ± 0.4	2097
35	OSI-10206	2.0 ± 0.0	7.0 ± 1.0	2.5 ± 0.5	9.0 ± 1.0	2983

36	OSI-10268	No effect	No effect	No effect	-	-
37	OSI-10173	0.6 ± 0.1	No effect	0.6 ± 0.1	> 100	> 2000
38	OSI-10269	No effect	No effect	No effect	-	-
39	OSI-10248	22.0 ± 0.5	No effect	3.0 ± 0.3	No effect	> 2000
40	OSI-10281	3.4 ± 0.4	No effect	0.4 ± 0.1	No effect	> 2000
41	OSI-10249	10.5 ± 0.7	No effect	0.8 ± 0.2	18.0 ± 1.4	> 2000
42	OSI-10033	20.4 ± 0.1	24.8 ± 1.2	2.3 ± 0.4	18.0 ± 1.0	2011
43	OSI-10279	No effect	No effect	No effect	-	-
44	OSI-20280	No effect	No effect	No effect	-	-
45	OSI-10000	No effect	No effect	No effect	-	959
46	OSI-10291	No effect	No effect	No effect	-	-
47	OSI-10183	31.4 ± 0.8	No effect	14.5 ± 3.5	-	> 2000
48	OSI-10184	32.5 ± 2.1	No effect	No effect	No effect	3084
49	OSI-10272	No effect	No effect	No effect	-	-

The following examples are illustrating but not restricting the present invention.

Reagents and solvents used below can be obtained from commercial sources. ^1H NMR spectra were recorded on a Varian Mercury 200 MHz and Varian Mercury 400 MHz NMR spectrometr. The ^1H chemical shifts are reported in ppm relative to HMDSO. Significant peaks are tabulated in the order: number of protons and multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; qui, quintet; se, sextet, m, multiplet; br.s, broad singlet), coupling constant(s) in Hertz (Hz). Melting points were determined on OptiMelt MPA100 apparatus and are uncorrected.

10 **General method for preparation of thieno[2,3-*b*]pyridines (I-1) from intermediates (II-1)**



Reagents and conditions :1) 2.4 equiv 3M NaOH/H₂O, rt, 10 min, EtOH;
2) 2-bromoacetophenone, 78 °C, 2-3 min, then rt, 30 min, 39-96%

To solution of 5-acetyl-4-(3,4-dimethoxyphenyl)-6-methyl-2-thioxo-1,6-dihydropyridine-3-carbonitrile (**50**) (0.16 g, 0.5 mmol) in 10 ml of ethanol 3M sodium hydroxide water solution (0.4 ml, 1.2 mmol) was added. The reaction mixture was shortly refluxed to form thiolate. Then 2-bromo-4'-methoxyacetophenone (0.11 g, 0.5 mmol) was added, the reaction mixture was refluxed for 5 min and stirred at room temperature for 30 min. The precipitated crystals were separated by filtration and purified by washing with ethanol and water during the filtration to give 0.21 g (91%) of 5-acetyl-3-amino-4-(3,4-dimethoxyphenyl)-2-(4-methoxybenzoyl)-6-methylthieno[2,3-*b*]pyridine (**1**) (general formula (I-1)).

In a similar manner thieno[2,3-*b*]pyridines **2-40** (general formula (I-1)) have been prepared starting from original 6-thioxo-1,6-dihydropyridines (yield, melting points and ^1H NMR data are combined in tables 4 and 5) or from 6-thioxo-1,6-dihydropyridines prepared according to literature data. The yields, melting points and ^1H NMR data of thieno[2,3-*b*]pyridines (general formula (I-1)) are combined in tables 4 and 5.

Table 4. Yields (general procedure) and melting points of thieno[2,3-*b*]pyridines (**I-1**).

Compound No.	Compound name	R ¹	R ²	R ⁶	R ⁷	Yield, %	Mp, °C
1	OSI-10036	Me	COMe	3,4-(OMe) ₂	4-OMe	91	176-178
2	OSI-10039	Me	COMe	3,4-(OMe) ₂	2,4-(OMe) ₂	85	182-184
3	OSI-10125	Me	COMe	3,4,5-(OMe) ₃	4-OMe	72	162-164
4	OSI-10120	Me	COMe	3,4,5-(OMe) ₃	2,4-(OMe) ₂	61	160-162
5	OSI-10215	Me	COOMe	4-OMe	4-OMe	78	179-180
6	OSI-9998	Me	COOMe	3,4-(OMe) ₂	4-OMe	84	192-194
7	OSI-10106	Me	COOMe	3,4-(OMe) ₂	2,4-(OMe) ₂	42	168-169
8	OSI-10105	Me	COOMe	3,4,5-(OMe) ₃	4-F	89	195-196
9	OSI-10104	Me	COOMe	3,4,5-(OMe) ₃	4-Cl	87	190-191
10	OSI-10103	Me	COOMe	3,4,5-(OMe) ₃	4-OMe	74	190-191
11	OSI-10102	Me	COOMe	3,4,5-(OMe) ₃	2,4-(OMe) ₂	87	138-139
12	OSI-10185	Me	COOMe	3,4,5-(OMe) ₃	3,4,5-(OMe) ₃	86	197-198
13	OSI-10164	Me	COOEt	H	4-OMe	92	150-152
14	OSI-10301	Me	COOEt	4-OH	4-OMe	39	196-198
15	OSI-10237	Me	COOEt	3-OMe	4-OMe	88	134-136
16	OSI-10302	Me	COOEt	3-OMe, 4-OH	4-OMe	67	201-203
17	OSI-10214	Me	COOEt	4-OMe	4-OMe	86	150-151
18	OSI-9992	Me	COOEt	3,4-(OMe) ₂	4-F	92	204-206
19	OSI-9994	Me	COOEt	3,4-(OMe) ₂	4-Cl	90	178-180
20	OSI-9993	Me	COOEt	3,4-(OMe) ₂	4-OMe	92	188-190
21	OSI-9991	Me	COOEt	3,4-(OMe) ₂	2,4-(OMe) ₂	88	176-178
22	OSI-9999	Me	COOEt	3,4,5-(OMe) ₃	4-F	92	193-195
23	OSI-10034	Me	COOEt	3,4,5-(OMe) ₃	4-Cl	89	189-191
24	OSI-9995	Me	COOEt	3,4,5-(OMe) ₃	4-OMe	89	204-206
25	OSI-9767	Me	COOEt	3,4,5-(OMe) ₃	2,4-(OMe) ₂	96	174-176
26	OSI-10266	Me	COOEt	4-OEt	4-OMe	96	150-151
27	OSI-10257	Me	COOEt	4-OBu(<i>n</i>)	4-OMe	89	105-106
28	OSI-10241	Me	COOEt	4-Cl	4-OMe	93	139-140
29	OSI-10240	Me	COOEt	4-NMe ₂	4-OMe	91	170-171
30	OSI-10243	Me	COOBu(<i>n</i>)	3,4,5-(OMe) ₃	4-OMe	93	149-150
31	OSI-10276	Me	COOC ₂ H ₄ OMe	4-OMe	4-OMe	90	125-126
32	OSI-10267	Me	COOC ₂ H ₄ OMe	3,4,5-(OMe) ₃	H	90	100-101
33	OSI-10175	Me	COOC ₂ H ₄ OMe	3,4,5-(OMe) ₃	3-OMe	78	131-132
34	OSI-10205	Me	COOC ₂ H ₄ OMe	3,4,5-(OMe) ₃	4-OMe	82	154-155
35	OSI-10206	Me	COOC ₂ H ₄ OMe	3,4,5-(OMe) ₃	2,4-(OMe) ₂	92	129-131
36	OSI-10268	Me	COOC ₂ H ₄ OMe	3,4,5-(OMe) ₃	3,4,5-(OMe) ₃	81	172-173
37	OSI-10173	Me	COOC ₂ H ₄ OPr(<i>n</i>)	3,4,5-(OMe) ₃	4-OMe	77	79-80
38	OSI-10269	Me	COOC ₂ H ₄ OPr(<i>n</i>)	3,4,5-(OMe) ₃	3,4,5-(OMe) ₃	72	155-156
39	OSI-10248	3,4,5-(OMe) ₃ C ₆ H ₂	COOEt	3,4,5-(OMe) ₃	4-OMe	95	196-197
40	OSI-10281	3,4,5-(OMe) ₃ C ₆ H ₂	COOEt	3,4,5-(OMe) ₃	3,4,5-(OMe) ₃	90	178-179

¹H NMR (400 MHz) spectra of compounds of formula (**I**) which include compounds of

5 formula (**I-1**) and (**I-2**) are combined in Table 5.

Table 5. ^1H NMR spectra of thieno[2,3-*b*]pyridines (**I**)

Compound No.	Compound name	^1H NMR spectra of compounds of formula (I)
1	OSI-10036	(CDCl_3) δ 2.02 (3H, s, COMe), 2.60 (3H, s, Me); 3.88, 3.89 and 3.98 (3H, 3H and 3H, s, s and s, $\text{C}_6\text{H}_3(\text{OMe})_2$ and $\text{C}_6\text{H}_4\text{OMe}$); 6.76 (2H, br.s, NH_2), 6.88, 6.94-7.03, 7.87 (1H, 4H, 2H, d, $J=1.6$ Hz, m, d, $J=8.6$ Hz, $\text{C}_6\text{H}_3(\text{OMe})_2$ and $\text{C}_6\text{H}_4\text{OMe}$)
2	OSI-10039	(CDCl_3) δ 2.01 (3H, s, COMe); 2.58 (3H, s, Me); 3.82, 3.87, 3.89 and 3.98 (3H, 3H, 3H and 3H, s, s, s and s, $2\times\text{C}_6\text{H}_3(\text{OMe})_2$); 6.70 (2H, br.s, NH_2); 6.52-6.55, 6.87-7.02, 7.37 (2H, 3H and 1H, m, m and d, $J=8.6$ Hz, $2\times\text{C}_6\text{H}_3(\text{OMe})_2$)
3	OSI-10125	(CDCl_3) δ 2.06 (3H, s, COMe); 2.60 (3H, s, Me); 3.86, 3.88 and 3.95 (6H, 3H and 3H, s, s and s, $\text{C}_6\text{H}_4\text{OMe}$ and $\text{C}_6\text{H}_2(\text{OMe})_3$); 6.79 (2H, br.s, NH_2); 6.59, 6.97 and 7.87 (2H, 2H and 2H, s, d, $J=8.6$ Hz, and d, $J=8.6$ Hz, $\text{C}_6\text{H}_2(\text{OMe})_3$ and $\text{C}_6\text{H}_4\text{OMe}$)
4	OSI-10120	(CDCl_3) δ 2.05 (3H, s, COMe); 2.58 (3H, s, Me); 3.82, 3.87 and 3.95 (3H, 9H and 3H, s, s and s, $\text{C}_6\text{H}_3(\text{OMe})_2$ and $\text{C}_6\text{H}_2(\text{OMe})_3$); 6.77 (2H, br.s, NH_2); 6.53-6.55, 6.59 and 7.38 (2H, 2H and 1H, m, s, and d, $J=8.2$ Hz, $\text{C}_6\text{H}_2(\text{OMe})_3$ and $\text{C}_6\text{H}_3(\text{OMe})_2$)
5	OSI-10215	(CDCl_3) δ 2.67 (3H, s, Me); 3.58 (3H, s, COOMe); 3.88 and 3.90 (3H and 3H, s and s, $2\times\text{C}_6\text{H}_4\text{OMe}$); 6.68 (2H, br.s, NH_2); 6.97, 7.04, 7.32 and 7.86 (2H, 2H, 2H and 2H, d, d, d, and d, $J=8.6$ Hz, $2\times\text{C}_6\text{H}_4\text{OMe}$)
6	OSI-9998	(CDCl_3) δ 2.61 (3H, s, Me); 3.54 (3H, s, COOMe) 3.82, 3.83 and 3.91 (3H, 3H and 3H, s, s and s, $\text{C}_6\text{H}_3(\text{OMe})_2$ and $\text{C}_6\text{H}_4\text{OMe}$); 6.67 (2H, br.s, NH_2), 6.83-6.95, 6.91 and 7.80 (3H, 2H and 2H, m, d and d, $J=8.6$ Hz, $\text{C}_6\text{H}_3(\text{OMe})_2$ and $\text{C}_6\text{H}_4\text{OMe}$)
7	OSI-10106	(CDCl_3) δ 2.58 (3H, s, Me); 3.53 (3H, s, COOMe); 3.75, 3.80, 3.83 and 3.91 (3H, 3H, 3H and 3H, s, s, s, and s, $2\times\text{C}_6\text{H}_3(\text{OMe})_2$); 6.45-6.48, 6.82-6.94 and 7.20 (2H, 3H and 1H, m, m and d, $J=8.6$ Hz, $2\times\text{C}_6\text{H}_3(\text{OMe})_2$); 6.64 (2H, br.s, NH_2)
8	OSI-10105	(CDCl_3) δ 2.62 (3H, s, Me); 3.56 (3H, s, COOMe); 3.80 and 3.90 (6H and 3H, s and s, $\text{C}_6\text{H}_2(\text{OMe})_3$); 6.54, 7.07-7.12, and 7.82 (2H, 2H and 2H, s, m and m, $\text{C}_6\text{H}_2(\text{OMe})_3$ and $\text{C}_6\text{H}_4\text{F}$); 6.80 (2H, br.s, NH_2)
9	OSI-10104	(CDCl_3) δ 2.61 (3H, s, Me); 3.56 (3H, s, COOMe); 3.80 and 3.90 (6H and 3H, s and s, $\text{C}_6\text{H}_2(\text{OMe})_3$); 6.54, 7.40 and 7.73 (2H, 2H and 2H, s, d and d, $J=8.6$ Hz, $\text{C}_6\text{H}_2(\text{OMe})_3$ and $\text{C}_6\text{H}_4\text{Cl}$); 6.78 (2H, br.s, NH_2)
10	OSI-10103	(CDCl_3) δ 2.61 (3H, s, Me); 3.56 (3H, s, COOMe); 3.80, 3.82 3.88 (6H and 3H, 3H, s, s and s, $\text{C}_6\text{H}_2(\text{OMe})_3$ and $\text{C}_6\text{H}_4\text{OMe}$); 6.54, 6.90 and 7.81 (2H, 2H and 2H, s, d and d, $J=8.2$ Hz, $\text{C}_6\text{H}_2(\text{OMe})_3$ and $\text{C}_6\text{H}_4\text{OMe}$); 6.70 (2H, br.s, NH_2)
11	OSI-10102	(CDCl_3) δ 2.58 (3H, s, Me); 3.56 (3H, s, COOMe); 3.75 3.80 and 3.87 (3H, 9H and 3H, s, s and s, $\text{C}_6\text{H}_3(\text{OMe})_2$ and $\text{C}_6\text{H}_2(\text{OMe})_3$); 6.46-6.48, 6.53 and 7.31 (2H, 2H and 1H, m, s and d $J=7.8$ Hz, $\text{C}_6\text{H}_3(\text{OMe})_2$ and $\text{C}_6\text{H}_2(\text{OMe})_3$); 6.77 (2H, br.s, NH_2)
12	OSI-10185	(CDCl_3) δ 2.67 (3H, s, Me), 3.61 (3H, s, COOMe); 3.85, 3.91 and 3.94 (6H, 9H, 3H, s, s and s, $\text{C}_6\text{H}_2(\text{OMe})_3$ and $\text{C}_6\text{H}_2(\text{OMe})_3$); 6.59 and 7.14 (2H and 2H, s and s, $\text{C}_6\text{H}_2(\text{OMe})_3$ and $\text{C}_6\text{H}_2(\text{OMe})_3$); 6.78 (2H, br.s, NH_2)
13	OSI-10164	(CDCl_3) δ 0.94 and 4.01 (3H and 2H, t and q, $J=7.0$ Hz, COOEt); 2.70 (3H, s, Me); 3.88 (3H, s, $\text{C}_6\text{H}_4\text{OMe}$); 6.57 (2H, br.s, NH_2); 6.97, 7.41-7.54 and 7.86 (2H, 5H and 2H, d, $J=8.6$ Hz, m, d, $J=8.6$ Hz, $\text{C}_6\text{H}_4\text{OMe}$ and C_6H_5)
14	OSI-10301	($\text{DMSO}-d_6$) δ 0.89 and 3.98 (5H, t and q, $J=7.0$ Hz, COOEt); 2.54 (3H, s, Me); 3.81 (3H, s, $\text{C}_6\text{H}_4\text{OMe}$); 6.69 (2H, s, NH_2); 6.90, 7.04, 7.18 and 7.73 (2H, 2H, 2H and 2H, d, d, d and d, $J=8.6$ Hz, $\text{C}_6\text{H}_4\text{OH}$ and $\text{C}_6\text{H}_4\text{OMe}$); 9.97 (1H, s, $\text{C}_6\text{H}_4\text{OH}$).
15	OSI-10237	(CDCl_3) δ 0.99 and 4.05 (3H and 2H, t and q, $J=7.0$ Hz, COOEt); 2.70 (3H, s, Me); 3.84 and 3.88 (3H and 3H, s and s, $2\times\text{C}_6\text{H}_4\text{OMe}$); 6.70 (2H, br.s, NH_2); 6.92, 6.96-6.99, 7.06, 7.43, 7.86 (1H, 3H, 1H, 1H and 2H, t, $J=2.3$ Hz, m, dd, $J=2.3$ Hz, $J=8.2$ Hz, t, $J=8.2$ Hz, d, $J=8.6$ Hz, $2\times\text{C}_6\text{H}_4\text{OMe}$)
16	OSI-10302	($\text{DMSO}-d_6$) δ 0.91 and 4.00 (5H, t and q, $J=7.0$ Hz, COOEt); 2.19 (3H, s, Me); 3.70 and 3.84 (3H and 3H, s and s, $\text{C}_6\text{H}_4\text{OMe}$ and $\text{C}_6\text{H}_3\text{OMe}$); 6.74 (2H, s, NH_2), 6.87-6.93, 7.06 and 8.04 (3H, 2H and 2H, m, d and d, $J=9.0$ Hz, $\text{C}_6\text{H}_3\text{OMeOH}$ and

		<u>C₆H₄OMe</u>)
17	OSI-10214	(CDCl ₃) δ 1.02 and 4.06 (3H and 2H, t and q, <i>J</i> =7.0 Hz, COOEt); 2.68 (3H, s, Me); 3.88 and 3.89 (3H and 3H, s and s, 2x <u>C₆H₄OMe</u>); 6.67 (2H, br.s, NH ₂); 6.97, 7.04, 7.33 and 7.86 (2H, 2H, 2H and 2H, d, d, d and d, <i>J</i> =8.6 Hz, 2x <u>C₆H₄OMe</u>)
18	OSI-9992	(CDCl ₃) δ 0.97 and 4.01 (3H and 2H, t and q, <i>J</i> =7.0 Hz, COOEt); 2.62 (3H, s, Me); 3.83 and 3.91 (3H and 3H, s and s, <u>C₆H₃(OMe)₂</u>); 6.70 (2H, br.s, NH ₂); 6.90-6.97, 6.99, 7.15 and 7.84-7.87(1H, 2H, 2H and 2H, m, t, t, <i>J</i> =8.6 Hz and m, <u>C₆H₃(OMe)₂</u> and <u>C₆H₄F</u>)
19	OSI-9994	(CDCl ₃) δ 0.97 and 4.01 (3H and 2H, t and q, <i>J</i> =7.0 Hz, COOEt); 2.62 (3H, s, Me); 3.83 and 3.91 (3H and 3H, s and s, <u>C₆H₃(OMe)₂</u>); 6.70 (2H, br.s, NH ₂); 6.83-6.95, 7.38 and 7.71 (3H, 2H and 2H, m, d and d, <i>J</i> =8.6 Hz, <u>C₆H₃(OMe)₂</u> and <u>C₆H₄Cl</u>)
20	OSI-9993	(CDCl ₃) δ 0.97 and 4.01 (3H and 2H, t and q, <i>J</i> =7.0 Hz, COOEt); 2.62 (3H, s, Me); 3.82 (3H and 3H, s and s, <u>C₆H₃(OMe)₂</u>); 3.91 (3H, s, <u>C₆H₄OMe</u>) 6.64 (2H, br.s, NH ₂); 6.84-6.93, 6.92 and 7.80 (3H, 2H and 2H, m, d and d, <i>J</i> =8.6 Hz, <u>C₆H₃(OMe)₂</u> and <u>C₆H₄OMe</u>)
21	OSI-9991	(CDCl ₃) δ 0.96 and 3.89 (3H and 2H, t and q, <i>J</i> =7.0 Hz, COOEt); 2.60 (3H, s, Me); 3.75, 3.80, 3.83 and 3.90 (3H, 3H, 3H and 3H, s, s, s and s, 2x <u>C₆H₃(OMe)₂</u>); 6.46-6.48, 6.83-6.92 and 7.30-7.32 (3H, 3H and 2H, m, m and m, 2x <u>C₆H₃(OMe)₂</u> and NH ₂)
22	OSI-9999	(CDCl ₃) δ 0.96 and 4.03 (3H and 2H, t and q, <i>J</i> =7.0 Hz, COOEt); 2.63 (3H, s, Me); 3.80 and 3.87 (6H and 3H, s and s, <u>C₆H₂(OMe)₃</u>); 6.55, 7.09 and 7.80 (2H, 2H and 2H, s, t and t, <i>J</i> =8.6 Hz, <u>C₆H₂(OMe)₃</u> and <u>C₆H₄F</u>), 6.77 (2H, br.s, NH ₂)
23	OSI-10034	(CDCl ₃) δ 0.96 and 4.03 (3H and 2H, t and q, <i>J</i> =7.0 Hz, COOEt); 2.63 (3H, s, Me); 3.80 and 3.87 (6H and 3H, s and s, <u>C₆H₂(OMe)₃</u>); 6.55, 6.91 and 7.80 (2H, 2H and 2H, s, d and d, <i>J</i> =8.6 Hz, <u>C₆H₂(OMe)₃</u> and <u>C₆H₄Cl</u>), 6.79 (2H, br.s, NH ₂)
24	OSI-9995	(CDCl ₃) δ 0.96 and 4.02 (3H and 2H, t and q, <i>J</i> =7.0 Hz, COOEt); 2.63 (3H, s, Me); 3.80, 3.83 and 3.87 (6H, 3H and 3H, s, s and s, <u>C₆H₂(OMe)₃</u> and <u>C₆H₄OMe</u>); 6.55, 6.91 and 7.80 (2H, 2H and 2H, s, d and d, <i>J</i> =8.6 Hz, <u>C₆H₂(OMe)₃</u> and <u>C₆H₄OMe</u>), 6.70 (2H, s, NH ₂)
25	OSI-9767	(CDCl ₃) δ 0.95 and 4.01 (3H and 2H, t and q, <i>J</i> =7.0 Hz, COOEt); 2.60 (3H, s, Me); 3.75, 3.80 and 3.86 (3H, 9H and 3H, s, s and s, <u>C₆H₂(OMe)₃</u> and <u>C₆H₃(OMe)₂</u>); 6.45-6.54 and 7.29-7.33 (2H and 3H, m and m, <u>C₆H₂(OMe)₃</u> and <u>C₆H₃(OMe)₂</u>)
26	OSI-10266	(CDCl ₃) δ 1.02 and 4.06 (3H and 2H, t and q, <i>J</i> =7.4 Hz, <u>C₆H₄OEt</u>); 1.48 and 4.11 (3H and 2H, t and q, <i>J</i> =7.0 Hz, COOEt); 2.68 (3H, s, Me); 3.88 (3H, s, <u>C₆H₄OMe</u>); 6.66 (2H, br.s, NH ₂); 6.97, 7.02, 7.31 and 7.86 (2H, 2H, 2H and 2H, d, d, d and d, <i>J</i> =8.6 Hz, <u>C₆H₄OMe</u> and <u>C₆H₄OEt</u>)
27	OSI-10257	(CDCl ₃) δ 0.99-1.04, 1.49-1.59, 1.79-1.86 and 4.02-4.09 (6H, 2H, 2H and 4H, m, m, m, m, COOEt and <u>C₆H₄OBu</u>); 2.68 (3H, s, Me); 3.88 (3H, s, <u>C₆H₄OMe</u>); 6.67 (2H, br.s, NH ₂); 6.97, 7.02, 7.31 and 7.86 (2H, 2H, 2H and 2H, d, d, d and d, <i>J</i> =8.6 Hz, <u>C₆H₄OMe</u> and <u>C₆H₄OBu</u>)
28	OSI-10241	(CDCl ₃) δ 1.02 and 4.06 (3H and 2H, t and q, <i>J</i> =7.0 Hz, COOEt); 2.70 (3H, s, Me); 3.89 (3H, s, <u>C₆H₄OMe</u>); 6.54 (2H, br.s, NH ₂); 6.97, 7.37, 7.52 and 7.86 (2H, 2H, 2H and 2H, d, d, d and d, <i>J</i> =8.6 Hz, <u>C₆H₄OMe</u> and <u>C₆H₄Cl</u>)
29	OSI-10240	(CDCl ₃) δ 1.04 and 4.08 (3H and 2H, t and q, <i>J</i> =7.0 Hz, COOEt); 2.67 (3H, s, Me); 3.04 (6H, s, NMe ₂); 3.88 (3H, s, <u>C₆H₄OMe</u>); 6.78, 6.96, 7.23 and 7.86 (2H, 2H, 2H and 2H, d, d, d and d, <i>J</i> =8.6 Hz, <u>C₆H₄OMe</u> and <u>C₆H₄NMe₂</u>); 6.82 (2H, br.s, NH ₂)
30	OSI-10243	(CDCl ₃) δ 0.85, 1.20, 1.38 and 4.02 (3H, 2H, 2H and 2H, t, se, <i>J</i> =7.4 Hz, qui, t, <i>J</i> =6.7 Hz, COOBu); 2.69 (3H, s, Me); 3.86, 3.89 and 3.94 (6H, 3H and 3H, s, s and s, <u>C₆H₂(OMe)₃</u> and <u>C₆H₄OMe</u>); 6.61, 6.97 and 7.87 (2H, 2H and 2H, s, d and d, <i>J</i> =8.6 Hz, <u>C₆H₄(OMe)</u> and <u>C₆H₂(OMe)₃</u>); 6.77 (2H, br.s, NH ₂)
31	OSI-10276	(CDCl ₃) δ 2.69 (3H, s, Me); 3.30, 3.36 and 4.15 (3H, 2H and 2H, s, t and t, <i>J</i> =4.7 Hz COO(CH ₂) ₂ OMe); 3.88 and 3.90 (3H and 3H, s and s, 2x <u>C₆H₄OMe</u>); 6.64 (2H, br.s, NH ₂); 6.97; 7.04, 7.34 and 7.86 (2H, 2H, 2H and 2H, d, d, d and d, <i>J</i> =8.6 Hz, 2x <u>C₆H₄OMe</u>)
32	OSI-10267	(CDCl ₃) δ 2.69 (3H, s, Me); 3.28, 3.35 and 4.17 (3H, 2H and 2H, s, t and t, <i>J</i> =4.7 Hz COO(CH ₂) ₂ OMe); 3.87 and 3.95 (6H and 3H; s and s, <u>C₆H₂(OMe)₃</u>); 6.62,

		7.46-7.55 and 7.83 (2H, 3H and 2H, s, m and d, $J=8.2$ Hz, $\underline{\text{C}_6\text{H}_2(\text{OMe})_3}$ and C_6H_5); 6.82 (2H, br.s, NH_2)
33	OSI-10175	(CDCl_3) δ 2.70 (3H, s, Me); 3.28, 3.35 and 4.17 (3H, 2H and 2H, s, t and t, $J=4.7$ Hz, $\text{COO}(\text{CH}_2)_2\text{OMe}$); 3.87 and 3.95 (9H and 3H, s and s, $\underline{\text{C}_6\text{H}_2(\text{OMe})_3}$ and $\underline{\text{C}_6\text{H}_4\text{OMe}}$); 6.62, 7.06-7.08 and 7.34-7.44 (2H, 1H and 3H, s, m and m, $\underline{\text{C}_6\text{H}_4\text{OMe}}$ and $\underline{\text{C}_6\text{H}_2(\text{OMe})_3}$)
34	OSI-10205	(CDCl_3) δ 2.70 (3H, s, Me), 3.28, 3.35 and 4.17 (3H, 2H and 2H, s, t and t, $J=4.7$ Hz, $\text{COO}(\text{CH}_2)_2\text{OMe}$), 3.87, 3.89 and 3.95 (6H, 3H and 3H, s, s and s, $\underline{\text{C}_6\text{H}_2(\text{OMe})_3}$ and $\underline{\text{C}_6\text{H}_4\text{OMe}}$), 6.62, 6.97 and 7.87 (2H, 2H and 2H, s, d and d, $J=8.6$ Hz $\underline{\text{C}_6\text{H}_2(\text{OMe})_3}$ and $\underline{\text{C}_6\text{H}_4\text{OMe}}$), 6.78 (2H, br.s, NH_2)
35	OSI-10206	(CDCl_3) δ 2.67 (3H, s, Me), 3.2, 3.27 and 4.16 (3H, 2H and 2H, s, t and t, $J=4.7$ Hz, $\text{COO}(\text{CH}_2)_2\text{OMe}$), 3.82, 3.87 and 3.94 (3H, 9H and 3H, s, s and s, $\underline{\text{C}_6\text{H}_2(\text{OMe})_3}$ and $\underline{\text{C}_6\text{H}_3(\text{OMe})_2}$), 6.52-6.55, 6.60 and 7.38 (2H, 2H and 1H, m, s and d, $J=8.2$ Hz, $\underline{\text{C}_6\text{H}_3(\text{OMe})_2}$ and $\underline{\text{C}_6\text{H}_2(\text{OMe})_3}$) 6.72 (2H, br.s, NH_2)
36	OSI-10268	(CDCl_3) δ 2.71 (3H, s, Me); 3.29, 3.55 and 4.18 (3H, 2H and 2H, s, t and t, $J=4.7$ Hz, $\text{COO}(\text{CH}_2)_2\text{OMe}$); 3.87, 3.92 and 3.95 (6H, 9H and 3H, s, s and s, $2\times\underline{\text{C}_6\text{H}_2(\text{OMe})_3}$); 6.62 and 7.15 (2H and 2H, s and s, $2\times\underline{\text{C}_6\text{H}_2(\text{OMe})_3}$)
37	OSI-10173	(CDCl_3) δ 0.89, 1.51-1.59, 3.33, 3.41 and 4.17 (3H, 2H, 2H, 2H and 2H, t, $J=7.4$ Hz, m, t, $J=6.7$ Hz, t and t, $J=4.7$ Hz, $\text{COO}(\text{CH}_2)_2\text{OPr}$); 2.70 (3H, s, Me); 3.86, 3.89 and 3.95 (6H, 3H and 3H, s, s and s, $\underline{\text{C}_6\text{H}_2(\text{OMe})_3}$ and $\underline{\text{C}_6\text{H}_4\text{OMe}}$); 6.62, 6.97 and 7.87 (2H, 2H and 2H, s, d and d, $J=8.6$ $\underline{\text{C}_6\text{H}_2(\text{OMe})_3}$ and $\underline{\text{C}_6\text{H}_4\text{OMe}}$); 6.78 (2H, br.s, NH_2)
38	OSI-10269	(CDCl_3) δ 0.89, 1.51-1.60, 3.34, 3.42 and 4.18 (3H, 2H, 2H, 2H and 2H, t, $J=7.4$ Hz, m, t, $J=6.7$ Hz, t and t, $J=4.7$ Hz, $\text{COO}(\text{CH}_2)_2\text{OPr}$); 2.71 (3H, s, Me); 3.87, 3.93 and 3.95 (6H, 9H, 3H, s, s and s, $2\times\underline{\text{C}_6\text{H}_2(\text{OMe})_3}$); 6.62 and 7.15 (2H and 2H, s and s, $2\times\underline{\text{C}_6\text{H}_2(\text{OMe})_3}$); 6.86 (2H, br.s, NH_2)
39	OSI-10248	(CDCl_3) δ 0.87 and 3.94 (3H and 2H, t and q, $J=7.4$ Hz, COOEt); 3.86, 3.87, and 3.89 (9H, 9H and 3H, br.s, br.s and s, $2\times\underline{\text{C}_6\text{H}_2(\text{OMe})_3}$ and $\underline{\text{C}_6\text{H}_4\text{OMe}}$); 6.65, 6.96, 6.98 and 7.87 (2H, 2H, 2H and 2H, s, s, d and d, $J=9.0$ Hz, $2\times\underline{\text{C}_6\text{H}_2(\text{OMe})_3}$ and $\underline{\text{C}_6\text{H}_4\text{OMe}}$); 6.75 (2H, br.s, NH_2)
40	OSI-10281	(CDCl_3) δ 0.83 and 3.94 (3H and 2H, t and q, $J=7.0$ Hz, COOEt); 3.59, 3.86, 3.87, 3.88, 3.92 and 3.93 (6H, 3H, 6H, 9H and 3H, br.s, br.s, br.s, br.s and br.s, $3\times\underline{\text{C}_6\text{H}_2(\text{OMe})_3}$); 6.65, 6.95 and 7.15 (2H, 2H and 2H, s, s and s, $3\times\underline{\text{C}_6\text{H}_2(\text{OMe})_3}$); 6.80 (2H, br.s, NH_2)
41	OSI-10249	(CDCl_3) δ 2.66 (3H, s, Me); 3.62 (3H, s, COOMe); 3.86 and 3.94 (6H and 3H, s and s, $\underline{\text{C}_6\text{H}_2(\text{OMe})_3}$); 6.61 (2H, s, $\underline{\text{C}_6\text{H}_2(\text{OMe})_3}$); 7.56-7.60, 7.87-7.94 and 8.39 (2H, 4H and 1H, m, m and s, C_{10}H_7); 6.76 (2H, br.s, NH_2)
42	OSI-10033	(CDCl_3) δ 1.09 and 4.12 (3H and 2H, t and q, $J=7.0$ Hz, COOEt); 2.68 (3H, s, CH_3); 3.88 (3H, s, $\underline{\text{C}_6\text{H}_4\text{OMe}}$); 6.09 (2H, d, $J=7.4$ Hz, $\underline{\text{C}_6\text{H}_3(\text{O}_2\text{CH}_2)}$); 6.73 (2H, br.s, NH_2); 6.85-6.98, 7.86 (7H, m, d, $J=8.6$ Hz, $\underline{\text{C}_6\text{H}_3(\text{O}_2\text{CH}_2)}$ and $\underline{\text{C}_6\text{H}_4(\text{OMe})}$)
43	OSI-10279	($\text{DMSO}-d_6$) δ 1.08 and 4.14 (5H, t and q, $J=7.0$ Hz, COOEt); 2.57 (3H, s, CH_3); 3.83 (3H, s, $\underline{\text{C}_6\text{H}_4\text{OMe}}$); 6.76 and 6.77, 6.87 and 8.02 (1H, 1H and 1H, dd, $J=1.95$ Hz, d, $J=2.7$ Hz, d, $J=1.2$ Hz, $\text{C}_4\text{H}_5\text{O}$); 7.06 and 7.77 (2H and 2H, d and d, $J=9.0$ Hz, $\underline{\text{C}_6\text{H}_4\text{OMe}}$); 7.14 (2H, br.s, NH_2)
44	OSI-10280	($\text{DMSO}-d_6$) δ 3.72, 3.78 and 3.84 (3H, 6H and 3H, $\underline{\text{C}_6\text{H}_4\text{OMe}}$ and $\underline{\text{C}_6\text{H}_2(\text{OMe})_3}$); 6.89, 7.06 and 8.02 (2H, 2H and 2H, s, d and d, $J=9.0$ Hz, $\underline{\text{C}_6\text{H}_2(\text{OMe})_3}$ and $(\underline{\text{C}_6\text{H}_4\text{OMe})}$); 7.70 and 7.89 (2H and 2H, br.s and br.s, $2\times\text{NH}_2$)
45	OSI-10000	(CDCl_3) δ 2.51 (3H, s, 4-Me); 2.70 (3H, s, 6-Me); 3.74 and 3.80 (3H and 3H, s and s, $\underline{\text{C}_6\text{H}_3(\text{OMe})_2}$); 6.46, 6.47, 6.79, 7.33 (1H, 1H, 1H and 1H, s, s, s, d, 5-H, $\underline{\text{C}_6\text{H}_3(\text{OMe})_2}$), 7.14 (2H, s, NH_2)
46	OSI-10291	(CDCl_3) δ 2.67 (3H, s, Me); 3.87, 3.88, and 3.93 (3H, 6H and 3H, s, s and s, $\underline{\text{C}_6\text{H}_4\text{OMe}}$ and $\underline{\text{C}_6\text{H}_2(\text{OMe})_3}$); 6.63, 6.96 and 7.87 (2H, 2H and 2H, s, d and d, $J=9.0$ Hz, $\underline{\text{C}_6\text{H}_2(\text{OMe})_3}$ and $\underline{\text{C}_6\text{H}_4\text{OMe}}$); 6.90 (2H, br.s, NH_2); 6.98 (1H, s, 5-H)
47	OSI-10183	(CDCl_3) δ 3.91, and 3.96 (9H and 9H, s and s, $2\times\underline{\text{C}_6\text{H}_2(\text{OMe})_3}$); 6.69, 7.35, 7.44 and 7.81 (2H, 2H, 2H and 2H, s, s, d, $J=8.6$ Hz, d, $J=9.0$ Hz, $2\times\underline{\text{C}_6\text{H}_2(\text{OMe})_3}$ and $\underline{\text{C}_6\text{H}_4\text{Cl}}$); 6.95 (2H, br.s, NH_2); 7.50 (1H, s, 5-H)
48	OSI-10184	(CDCl_3 , δ , ppm): 3.83, 3.85, and 3.91 (3H, 9H and 9H, s, s and s, $\underline{\text{C}_6\text{H}_4\text{OMe}}$, $2\times\underline{\text{C}_6\text{H}_2(\text{OMe})_3}$); 6.64 6.92, 7.30 and 7.86 (2H, 2H, 2H, s, d, $J=8.6$ Hz, s, d, $J=9.0$ Hz $2\times\underline{\text{C}_6\text{H}_2(\text{OMe})_3}$, and $\underline{\text{C}_6\text{H}_4\text{OMe}}$); 6.84 (2H, br.s, NH_2); 7.30 (1H, s, 5-H)

49	OSI-10272	(CDCl ₃) δ 1.51 (3H, s, COMe); 2.73 (3H, s, Me); 3.28, 3.36 and 4.20 (3H, 2H and 2H, s, t and t, <i>J</i> =4.7 Hz, COO(CH ₂) ₂ OMe); 3.87, 3.88 and 3.90 (6H, 3H and 3H, s, s and s, C ₆ H ₂ (OMe) ₃ and C ₆ H ₄ OMe); 6.60, 6.94 and 7.94 (2H, 2H and 2H, s, d and d, <i>J</i> =8.6 Hz, C ₆ H ₂ (OMe) ₃ and C ₆ H ₄ OMe); 7.19 (1H, s, NH)
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Table 6. Synthesized 1,6-dihydro-6-thioxopyridines **II-1** as intermediates of MDR modulators **I**.

Compound No.	R ¹	R ²	R ⁶	Yield of II , %		Mp, °C
				Path A	Path B	
50	Me	COMe	3,4-(OMe) ₂	47		234-235
51	Me	COMe	3,4,5-(OMe) ₃	42		244-246
52	Me	COOMe	3,4-(OMe) ₂	42		230-232
53	Me	COOMe	3,4,5-(OMe) ₃	29	33	233-234
54	Me	COOEt	3-OMe		61	220-221
55	Me	COOEt	3-OMe,4-OH	29		206-208
56	Me	COOEt	4-OEt		54	184-185
57	Me	COOEt	4-OBu(<i>n</i>)		59	175-176
58	Me	COOBu(<i>n</i>)	3,4,5-(OMe) ₃		46	197-198
59	Me	COOC ₂ H ₄ OMe	4-OMe		53	210-211
60	Me	COOC ₂ H ₄ OMe	3,4,5-(OMe) ₃	42	53	192-194
61	Me	COOC ₂ H ₄ OPr	3,4,5-(OMe) ₃	57		140-141
62	3,4,5-(OMe) ₃ C ₆ H ₂	COOEt	3,4,5-(OMe) ₃		44	215-216
63	NH ₂	CN	3,4,5-(OMe) ₃		41	252-253

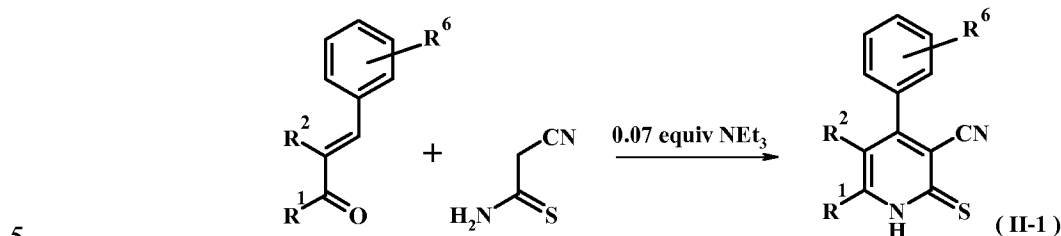
5

Table 7. ¹H NMR spectra of compounds of formula (**II-1**)

Compound No.	¹ H NMR spectra of 1,6-dihydro-6-thioxopyridines (II-1)
50	(CDCl ₃) δ 1.80 (3H, s, COMe); 2.50 (3H, s, Me); 3.90 and 3.95 (3H and 3H, s and s, C ₆ H ₃ (OMe) ₂); 6.92-7.00 (3H, m, C ₆ H ₃ (OMe) ₂); 12.53 (1H, br.s, NH)
51	(CDCl ₃) δ 1.83 (3H, s, COMe); 2.50 (3H, s, Me); 3.87 and 3.92 (3H and 6H, s and s, C ₆ H ₂ (OMe) ₃); 6.62 (2H, C ₆ H ₂ (OMe) ₃); 12.16 (1H, br.s, NH)
52	(CDCl ₃) δ 2.58 (3H, s, Me); 3.54 (3H, s, COOMe); 3.90 and 3.93 (3H and 3H, s and s, C ₆ H ₃ (OMe) ₂); 6.90-6.98 (3H, m, C ₆ H ₃ (OMe) ₂); 12.50 (1H, br.s, NH)
53	(CDCl ₃) δ 2.58 (3H, s, Me); 3.54 (3H, s, COOMe); 3.87 and 3.90 (6H and 3H, s and s, C ₆ H ₂ (OMe) ₃); 6.60 (2H, s, C ₆ H ₂ (OMe) ₃)
54	(CDCl ₃) δ 0.87 and 3.96 (3H and 2H, t and q, <i>J</i> =7.0 Hz, COOEt); 2.60 (3H, s, Me); 3.83 (3H, s, C ₆ H ₄ OMe); 6.88-7.40 (4H, m, C ₆ H ₄ OMe); 12.39 (1H, br.s, NH)
55	(CDCl ₃) δ 0.81 and 3.90 (5H, t and q, <i>J</i> =7.0, COOEt); 2.40 (3H, s, Me); 3.72 (3H, s, C ₆ H ₃ OMe); 6.73-6.89 (3H, m, C ₆ H ₃ OMe), 9.57 (1H, s, OH), 14.21 (1H, s, NH)
56	(CDCl ₃) δ 0.91 and 3.98 (3H and 2H, t and q, <i>J</i> =7.0 Hz, C ₆ H ₄ OEt); 1.44 and 4.07 (3H and 2H, t and q, <i>J</i> =7.0 Hz, COOEt); 2.57 (3H, s, Me); 6.97 and 7.31 (2H and 2H, d and d, <i>J</i> =8.6 Hz, C ₆ H ₄ OEt); 12.06 (1H, br.s, NH)
57	(CDCl ₃) δ 0.92, 0.99, 1.50, 1.79 and 3.97-4.02 (3H, 3H, 2H, 2H and 4H, t, se, <i>J</i> =7.0 Hz, qui, t, <i>J</i> =6.3 Hz, m, C ₆ H ₄ OBu and COOEt); 2.58 (3H, s, Me); 6.96 and 7.31 (2H and 2H, d and d, <i>J</i> =9.0 Hz, C ₆ H ₄ OBu); 12.41 (1H, br.s, NH)
58	(CDCl ₃) δ 0.79, 1.05, 1.25 and 3.92 (3H, 2H, 2H and 2H, t, se, <i>J</i> =7.0 Hz, qui and t, <i>J</i> =6.3 Hz, COOBu); 2.59 (3H, s, Me); 3.87 and 3.90 (3H and 6H, s and s, C ₆ H ₂ (OMe) ₃); 6.61 (2H, s, C ₆ H ₂ (OMe) ₃); 12.40 (1H, br.s, NH)

59	(CDCl ₃) δ 2.57 (3H, s, Me); 3.24-3.28 and 4.09 (5H and 2H, m and t, <i>J</i> =4.7 Hz, COO(CH ₂) ₂ OMe); 3.86 (3H, s, C ₆ H ₄ OMe); 6.99 and 7.34 (2H and 2H, d and d, <i>J</i> =8.6 Hz, C ₆ H ₄ OMe)
60	(CDCl ₃) δ 2.58 (3H, s, Me); 3.21-3.23 and 4.07 (5H and 2H, m and t, <i>J</i> =4.7 Hz, COO(CH ₂) ₂ OMe); 3.86 and 3.89 (6H and 3H, s and s, C ₆ H ₂ (OMe) ₃); 6.59 (2H, s, C ₆ H ₂ (OMe) ₃); 12.57 (1H, s, NH)
61	(CDCl ₃) δ 0.87, 1.5, 3.25-3.30, 4.10 (3H, 2H, 4H, 2H t, se, <i>J</i> =7 Hz m, t, <i>J</i> =4.7 Hz, COO(CH ₂) ₂ OPr); 2.17 (3H, s, Me); 3.87 and 3.90 (6H and 3H, s and s, C ₆ H ₂ (OMe) ₃); 6.61 (2H, s, C ₆ H ₂ (OMe) ₃); 12.71 (1H, br.s., NH)
62	(CDCl ₃) δ 0.87 and 4.00 and (3H and 2H, t and q, <i>J</i> =7.0 Hz, COOEt); 3.86 and 3.90 (9H and 9H, s and s, 2xC ₆ H ₂ (OMe) ₃); 6.57 and 6.88 (2H and 2H, s and s, 2xC ₆ H ₂ (OMe) ₃); 8.67 (1H, s, NH)
63	(CDCl ₃) δ 3.71 and 3.77 (6H and 3H, s and s, C ₆ H ₂ (OMe) ₃); 6.84 (2H, s and s, C ₆ H ₂ (OMe) ₃); 7.96 (2H, br.s, NH ₂); 8.86 (1H, s, NH)

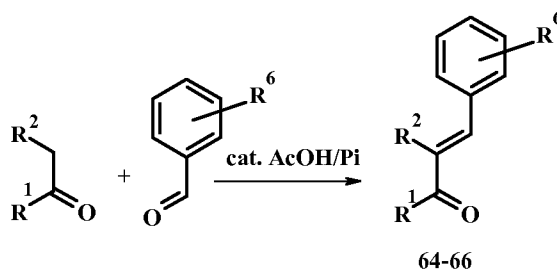
Preparation of the intermediates - 1,6-dihydro-6-thioxopyridines with general formula (II-1). Path A



10 A mixture of 3-(4,5-dimethoxybenzylidene)pentane-2,4-dione (1.24 g, 5 mmol), 2-cyanothioacetamide (0.50 g, 5 mmol) and triethylamine (0.05 ml, 0.35 mmol) in 20 ml of ethanol was refluxed for 15 h. The precipitated crystals were separated by filtration and purified by washing with ethanol and water during the filtration to give 0.77 g (47%) of 5-acetyl-4-(3,4-dimethoxyphenyl)-6-methyl-2-thioxo-1,2-dihydropyridine-3-carbonitrile (**50**) (general formula (II-1)) as yellow powder. In a similar manner 6-thioxo-1,6-dihydropyridines **51-53**, **55**, **60**, **61** (general formula (II-1)) have been prepared. The yields and melting points are combined in table 6, but ¹H NMR (400 MHz) spectra in table 7.

15

Preparation of the intermediates - α,β -unsaturated ketones 64-66



To a solution of 3-oxo-butyric acid 2-methoxy ethyl ester (8.00 g, 50 mmol) of and 3,4,5-trimethoxybenzaldehyde (9.81 g, 50 mmol) in 50 ml of 2-propanol, piperidine (0.35 ml, 3.5 mmol) and acetic acid (1.55 ml, 27 mmol) were added and reaction mixture was refluxed for one day. After solvent was evaporated, DCM was added and the reaction mixture was washed with NaHCO₃ aqueous solution. The organic layer was dried over anhydrous MgSO₄ and then concentrated under reduced pressure to give 14.21 g (84%) of *E,Z*-3-oxo-2-[1-(3,4,5-trimethoxyphenyl)methylidene]butyric acid 2-methoxy ethyl ester (**66**) as colourless powder with mp 92-93 °C. ¹H NMR (CDCl₃) δ 2.38 and 2.42 (0.5H and 2.5H, s and s, Me); 3.31 and 3.40, 3.62 and 3.70, 4.39 and 4.43 (2.5H and 0.5H, 1.7H and 0.3H, 0.3H and 1.7H, s and s, t and t, t and t, *J*=4.7 Hz, COO(CH₂)₂OMe); 3.84-3.89 (9H, m, C₆H₂(OMe)₃); 6.66 and 6.74 (0.3H and 1.7H, s and s, C₆H₂(OMe)₃); 7.49 and 7.61 (0.9H and 0.1H, s and s, 3-H).

In a similar manner α,β-unsaturated ketones **65** and **66** have been prepared.

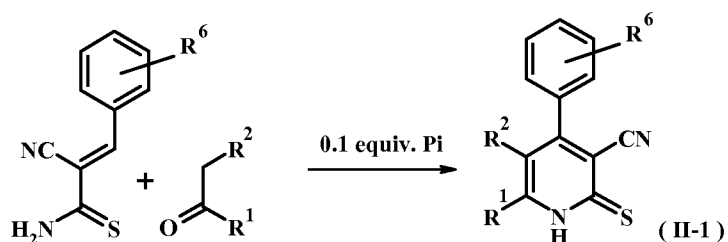
3-Oxo-2-[1-(3,4,5-trimethoxyphenyl)-methylidene]butyric acid 2-propoxy ethyl ester (65).

Yield 89%, mp 81-82 °C. ¹H NMR (CDCl₃) δ 2.42 (3H, s, Me); 0.84, 1.52, 3.35, 3.66 and 4.42 (3H, 2H, 2H, 2H and 2H, t, *J*=7.4 Hz, se, *J*=7.0 Hz, t, *J*=6.7 Hz, t, *J*=5.1 Hz, and t, *J*=5.1 Hz, COO(CH₂)₂OPr(*n*)); 3.86 and 3.89 (6H and 3H, s and s, C₆H₂(OMe)₃); 6.74 (2H, s and s, C₆H₂(OMe)₃); 7.49 (1H, s and s, 3-H).

2-(3,4,5-Trimethoxybenzoyl)-3-(3,4,5-trimethoxyphenyl)acrylic acid ethyl ester (66).

Yield 86%, mp 101-103 °C. ¹H NMR (CDCl₃) δ 1.24 and 4.26 (3H and 2H, t and q, *J* = 7.0 Hz, COOEt); 3.66, 3.81, 3.83 and 3.91 (6H, 3H, 6H and 3H, s, s, s and s, 2xC₆H₂(OMe)₃); 6.65 and 6.96 (2H and 2H, s and s, 2xC₆H₂(OMe)₃); 7.85 (1H, s, 3-H).

Preparation of the intermediates - 1,6-dihydro-6-thioxopyridines with general formula (II-1). Path B



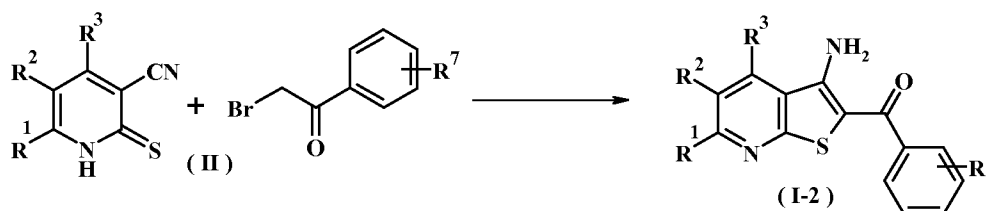
A mixture of 2-cyano-3-(4-ethoxyphenyl)thioacrylamide (1.16 g, 5 mmol), ethyl acetoacetate (0.637 ml, 5 mmol) and piperidine (0.05 ml, 0.5 mmol) in 25 ml of ethanol was refluxed for

15 h. The precipitated crystals were separated by filtration and purified by washing with ethanol and water during the filtration to give 1.04 g (61%) of 5-cyano-4-(4-ethoxyphenyl)-2-methyl-6-thioxo-1,6-dihydropyridine-3-carboxylic acid ethyl ester **54**.

In a similar manner 6-thioxo-1,6-dihydropyridines **53**, **56-60** and **62** (general formula (II-1)) have been prepared. The yields and melting points are combined in table 6, but ¹H NMR (400 MHz) spectra in table 7.

Preparation of thieno[2,3-*b*]pyridines (I-2) from intermediates (II) (examples 1-5).

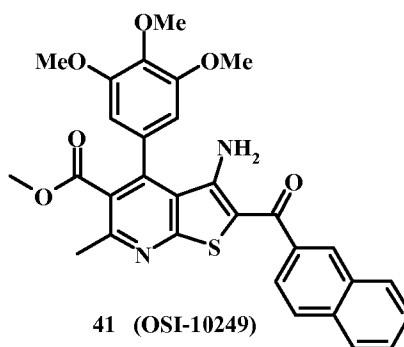
¹H NMR (400 MHz) spectra of thieno[2,3-*b*]pyridines **41-45** (general formula (I-2)) are combined in table 5.



Reagents and conditions :1) 2.4 equiv 3M NaOH/H₂O, rt, 10 min, EtOH;
2) 2-bromoacetophenone, 78 °C, 2-3 min, then rt, 30 min

15

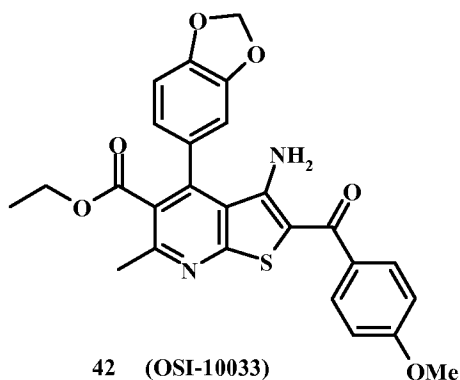
EXAMPLE 1



20 To a solution of 5-cyano-2-methyl-6-thioxo-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyridine-3-carboxylic acid methyl ester (**53**) (0.69 g, 2 mmol) in 10 ml of ethanol 3M sodium hydroxide water solution (1.60 ml, 4.8 mmol) was added and stirred at room temperature for 10 min. Then 2-bromo-1-naphthalen-2-yl-ethanone (0.50 g, 2 mmol) was

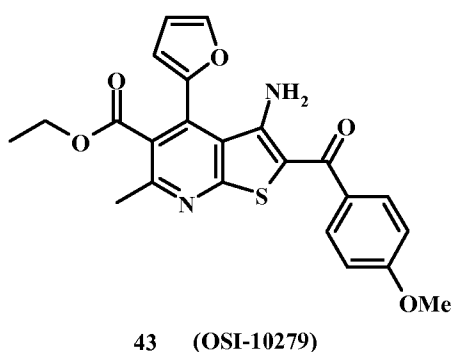
added and reaction mixture was shortly refluxed (0.5 min) and stirred for 30 min at room temperature. The precipitate was filtered and purified by washing with 3 ml of cold methanol and water during the filtration to give 0.97 g (89%) of 3-amino-6-methyl-2-(naphthalene-2-carbonyl)-4-(3,4,5-trimethoxyphenyl)thieno[2,3-*b*]pyridine-5-carboxylic acid methyl ester (41) as yellow powder with mp 150-151 °C.

EXAMPLE 2



10 To a solution of 4-(benzo[1,3]dioxol-5-yl)-5-cyano-2-methyl-6-thioxo-1,6-dihydropyridine-3-carboxylic acid ethyl ester (in Abunada et al., Phosphorus, Sulfur Silicon Rel Elem 3: 591-601 (2009)) (0.17 g, 0.5 mmol) in 5 ml of ethanol 3M sodium hydroxide water solution (0.4 ml, 1.2 mmol) was added and stirred at room temperature for 10 min. Then 2-bromo-4'-methoxyacetophenone (0.11 g, 0.5 mmol) was added and reaction mixture was shortly
15 refluxed and stirred at room temperature for 30 min. The precipitated crystals were separated by filtration and purified by washing with ethanol and water during the filtration to give 0.19 g (79%) of 3-amino-4-(benzo[1,3]dioxol-5-yl)-2-(4-methoxybenzoyl)-6-methylthieno[2,3-*b*]pyridine-5-carboxylic acid ethyl ester (42) as yellow powder with mp 168-170 °C.

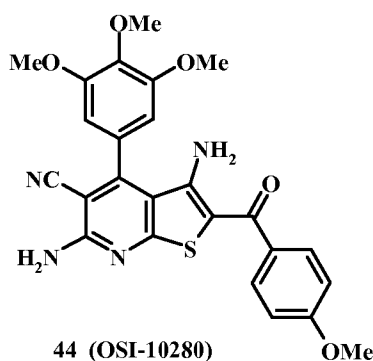
20 EXAMPLE 3



To a solution of 5-cyano-4-(furan-2-yl)-2-methyl-6-thioxo-1,6-dihydropyridine-3-carboxylic acid ethyl ester (in Elgemeie et al., Bull Chem Soc Jpn 12: 4431-34 (1988)) (0.30 g, 1 mmol) in 10 ml of ethanol 3M sodium hydroxide water solution (0.8 ml, 2.4 mmol) was added and stirred at room temperature for 10 min. Then 2-bromo-4'-methoxyacetophenone (0.24 g, 1 mmol) was added and reaction mixture was shortly refluxed and stirred at room temperature for 30 min. The precipitated crystals were separated by filtration and purified by washing with ethanol and water during the filtration to give 0.40 g (88%) 3-amino-4-(furan-2-yl)-2-(4-methoxybenzoyl)-6-methylthieno[2,3-*b*]pyridine-5-carboxylic acid ethyl ester (**43**) as yellow powder with mp 113-114 °C.

10

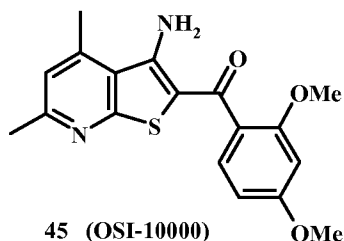
EXAMPLE 4



To a solution of 6-amino-2-thioxo-4-(3,4,5-trimethoxyphenyl)-1,2-dihydropyridine-3,5-dicarbonitrile (**63**) (0.34 g, 1 mmol) in 10 ml of ethanol 3M sodium hydroxide water solution (0.8 ml, 2.4 mmol) was added and stirred at room temperature for 10 min. Then 2-bromo-4'-methoxyacetophenone (0.24 g, 1 mmol) was added and reaction mixture was shortly refluxed and stirred at room temperature for 30 min. The precipitated crystals were separated by filtration and purified by washing with ethanol and water during the filtration to give 0.20 g (41%) of 3,6-diamino-2-(4-methoxybenzoyl)-4-(3,4,5-trimethoxyphenyl)thieno[2,3-*b*]pyridine-5-carbonitrile (**44**) as yellow powder with mp 241-242 °C.

20

EXAMPLE 5



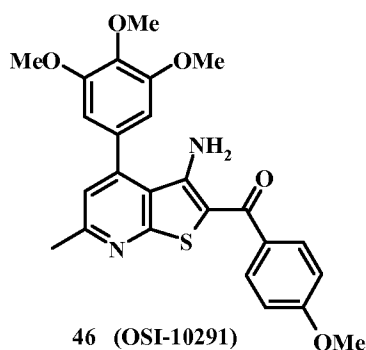
To a solution of 4,6-dimethyl-2-thioxo-1,2-dihydropyridine-3-carbonitrile (in Schmidt, Kubitzek, *Chemische Berichte* 93: 1559-1565 (1960)) (0.16 g, 1 mmol) in 10 ml of ethanol
5 3M sodium hydroxyde water solution (0.8 ml, 2.4 mmol) was added and stirred at room temperature for 10 min. Then 2-bromo-4'-methoxyacetophenone (0.24 g, 1 mmol) was added and reaction mixture was shortly refluxed and stirred at room temperature for 30 min. The precipitated crystals were separated by filtration and purified by washing with ethanol and water during the filtration to give 0.24 g (70%) 3-amino-4,6-dimethyl-2-(2,4-dimethoxybenzoyl)thieno[2,3-*b*]pyridine (**45**) as yellow powder with mp 184-186 °C.
10

Preparation of thieno[2,3-*b*]pyridines 46-49 (general formula (I)) (examples 6-9).

¹H NMR (400 MHz) spectra of compounds **46-49** (general formula (I)) are combined in table 5.

15

EXAMPLE 6

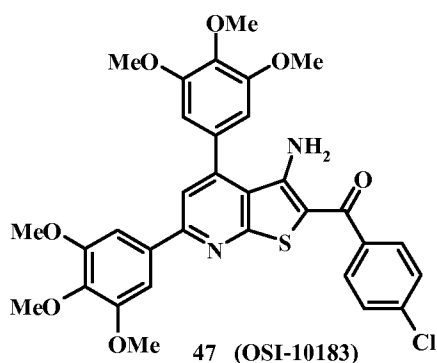


A mixture of 4-(3,4,5-trimethoxyphenyl)-3-buten-2-one (0.24 g, 1 mmol), 2-cyanothioacetamide (0.1 g, 1 mmol) and piperidine (0.11 ml, 1.1 mmol) in 10 ml of ethyl
20 alcohol was stirred at room temperature for 12 h. Then 2-bromo-1-(4-methoxyphenyl)ethanone (0.23 g, 1 mmol) was added and reaction mixture was shortly heated until reflux and stirred for 3 h at room temperature. Then 3M sodium hydroxide water solution (0.33 ml, 1 mmol) was added. Reaction mixture was refluxed for 1 min and stirred

at room temperature for 30 min. The precipitates was filtered and purified by washing with 1 ml of cold ethanol and water during the filtration to give 0.39 g (84%) of 3-amino-2-(4-methoxybenzoyl)-6-methyl-4-(3,4,5-trimethoxyphenyl)thieno[2,3-*b*]pyridine (**46**) as yellow powder with mp 234-235 °C.

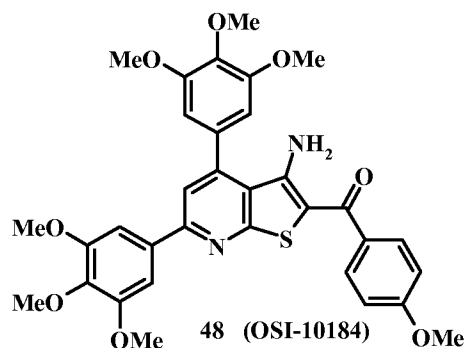
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EXAMPLE 7



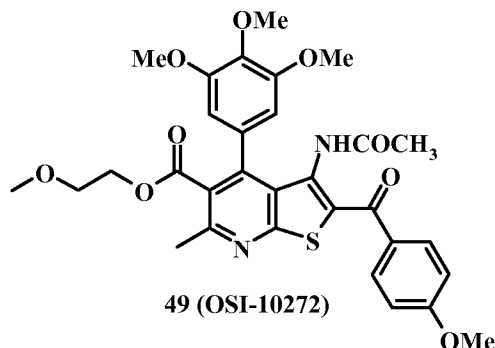
In a similar manner 3-amino-4,6-bis-(3,4,5-trimethoxyphenyl)-2-(4-chlorobenzoyl)thieno[2,3-*b*]pyridine (**47**) has been prepared. Yield 88%, mp 259-260 °C.

EXAMPLE 8



15 In a similar manner 3-amino-4,6-bis-(3,4,5-trimethoxyphenyl)-2-(4-methoxybenzoyl)-thieno[2,3-*b*]pyridine (**48**) has been prepared. Yield 84%, mp 243-244 °C.

EXAMPLE 9



To solution of 3-amino-2-(4-methoxybenzoyl)-6-methyl-4-(3,4,5-trimethoxyphenyl)thieno[2,3-*b*]pyridine-5-carboxylic acid 2-methoxyethyl ester (**34**) (OSI-10205) (0.19 g, 0.35 mmol) in 2.5 ml acetyl chloride NaOH (0.014g, 0.35 mmol) were added. The resulting mixture was refluxed for 2 h, cooled down, poured into iced water, neutralized with NaHCO₃ and extracted with DCM. Resulting extract was dried over MgSO₄ and evaporated. Oil was recrystallized using EtOH. The precipitated crystals were separated by filtration and purified by washing with ethanol and water during the filtration to give 0.15 g (71%) of 3-acetyl-amino-2-(4-methoxybenzoyl)-6-methyl-4-(3,4,5-trimethoxyphenyl)-thieno[2,3-*b*]pyridine-5-carboxylic acid 2-methoxyethyl ester (**49**) as yellow powder with mp 183-184 °C.

Biological studies of thieno[2,3-*b*]pyridines

15

The potency of P-glycoprotein-mediated MDR modulator *in vitro* was evaluated in the drug sensitive human sarcoma MES-SA cells and doxorubicin (DOX) resistant cells by accumulation of fluorescent substrate - rhodamine 123. Effects on MRP1-mediated drug efflux were observed in the DOX resistant human lung carcinoma H69AR cells using calcein AM assay. BCRP1-mediated drug efflux was also evaluated using fluorescent substrate Hoechst 33342 in the mitoxantrone resistant human sarcoma MESSA/MX2 cells. The half maximal effective concentrations (EC₅₀) were calculated from dose response curve as the most effective method to compare MDR-modulating activities of compounds. MDR modulating activity, intracellular Ca²⁺ values and LD₅₀ values of tested compounds of formula (**I**) are given in table 3.

25

Measurement of P-glycoprotein activity

About 50,000 cells/well of the human uterine sarcoma cell line MESSA and MES-SA/Dx5 were seeded into the 96-well plates and incubated for 24 h. The MDR modulators were incubated with the cells for 15 min. Then rhodamine-123 (2.6 μM) was added and the cells were incubated for 1h, 37 °C, 5% CO₂. The cells were washed twice with phosphate buffered saline (PBS) and fluorescence values were measured with excitation at 487 nm and emission at 557 nm by using the fluorescence reader Tecan infinite M1000. Verapamil, the known P-glycoprotein inhibitor was used as a positive control. The EC₅₀ values were calculated using the program Graph Pad Prism® 4.0.

10 **Measurement of MRP1 activity**

About 60,000 cells/well of the human lung carcinoma cell line H69 and H69AR were seeded into the 96-well plates and incubated for 24 h. The compounds were incubated with the cells for 15 min. Then Calcein AM (0.25 μM) was added and the cells were incubated for 30 min, 37 °C, 5% CO₂. The cells were washed twice with phosphate buffered saline (PBS) and fluorescence values were measured with excitation at 494 nm and emission at 517 nm by using the fluorescence reader Tecan infinite M1000. MK-571 was used as the known inhibitor of MRP1. The EC₅₀ values was calculated using the program Graph Pad Prism® 4.0

20

Measurement of BCRP activity

About 50,000 cells/well of the MESSA and MES-SA/MX2 were seeded into the 96-well plates and incubated for 24 h. The MDR modulators were incubated with the cells for 15 min. Hoechst 33342 (5 $\mu\text{g/ml}$) was added and the cells were incubated for 90 min, 37 °C, 5% CO₂. The cells were washed twice with phosphate buffered saline (PBS) and fluorescence values were measured with excitation at 360 nm and emission at 450 nm (Gail M.Seigel and Lorrie M. Campbell, 2004). Imatinib was used as a positive control. The EC₅₀ values were calculated using the program Graph Pad Prism® 4.0

30

Intracellular Ca²⁺ measurements

Many calcium channel blockers of all subclasses (phenylalkylamine, dihydropyridine and benzothiazepine type) and other calcium antagonists inhibit the P-gp - mediated drug

efflux. Calcium antagonists are overcoming P-gp - mediated MDR by mechanisms distinct from their inhibitory effect on voltage-dependent calcium channels. These mechanisms include not only direct interactions with the P-gp protein but also interactions with several other proteins that may indirectly alter P-gp transport activity. Calcium antagonists not only
5 can be inhibitors of P-gp but at the same time some may inhibit P-gp expression via P-gp mRNA down-regulation. The clinical application of calcium antagonists is limited by their severe cardiovascular side effects associated with the high concentration required for reversal of MDR. Hence, cardiotoxicity is a major complication of anticancer drugs including anthracyclines and 5-fluorouracils.

10 The effective MDR modulators with the less than ten times lower Ca^{2+} antagonist effect than verapamil, the known P-gp inhibitor, allow to decrease presumable cardiovascular side effects.

Changes in intracellular $[\text{Ca}^{2+}]_i$ concentration were studied using Fluo-4 NW Calcium Assay Kit (“Invitrogen”, Sweden) accordingly to manufacturer’s instructions. The
15 A7R5 (rat aorta smooth muscle) cells were grown at 37 °C in a humidified atmosphere with 5% CO_2 /95% air in DMEM medium containing 2 mM glutamine and supplemented with 10% FBS. The cells were seeded into 96 well plate at 10,000 cells/well and incubated for 72 h. The cells were loaded with Fura-4NW for 45 min. The Fura-4NW loaded cells were pre-incubated in the dark for 15 minutes with tested compounds at concentrations from 0.8 to
20 100 μM . Then A7R5 cells were pre-treated with 1.5 mM CaCl_2 for 5 minutes and KCl (50 mM) was added to A7R5 cells to induce $[\text{Ca}^{2+}]_i$ increase. Amlodipine, the well-known calcium channel inhibitor was used as the positive control. Changes in $[\text{Ca}^{2+}]_i$ were measured from the fluorescence emitted at 516 nm due to alternate excitation at 494 nm using the fluorescence spectrophotometer (Thermo Asciant, Finland). The IC_{50} values were
25 calculated using the program Graph Pad Prism® 4.0

Basal toxicity test

A low therapeutic index of chemotherapy agents is due to affecting not only cancer
30 formation but also normal cells. Compounds (I) were tested on NIH 3T3 (normal mouse fibroblasts, “ATCC”) cell line according the basal toxicity test (INVITOX Protocol No 64, 1992) and non-toxic compounds were selected.

9,000 NIH 3T3 cells/well were placed into 96-well plates for 24 h and then exposed to the test compound over a range of eight concentration (1-1000 $\mu\text{g/ml}$) for 24 h. Upon that,

the cells were incubated with the neutral red dye for 4 h and then OD was determined at 540 nm. Alternative LD₅₀ values (LD₅₀ value is the amount of the drug that is taken to kill 50% of the test animals) was calculated according to the formula: $\log(\text{LD}_{50} [\text{mmol/kg}] = 0.435 \times \log(\text{IC}_{50} [\text{mmol/l}]) = 0.625$. The IC₅₀ values were calculated using the program Graph Pad Prism® 4.0

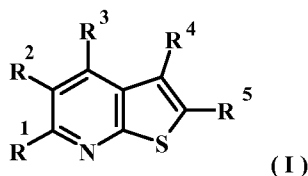
Cell culture

NIH3T3 (normal mouse fibroblasts), HT-1080 (human fibrosarcoma), MG-22A (mouse hepatoma), MES-SA (human uterine sarcoma), MES-SA/Dx5 (Doxorubicin resistant, 300 ng/ml), H69 (human lung carcinoma), H69/AR (Doxorubicin resistant) and A7R5 (rat aorta smooth muscle) cells were obtained from the ATCC cell collection.

MDR appearance is the process of activation of a number of gens and as a result of several proteins overexpression (48 human ABC transporters have been described, more probable MDR associated proteins are P-pg, MRP1 and BCRP).

Claims

1. Compounds of the general formula (I),



wherein

R^1 is Me, C_6H_5 , 3,4,5-(OMe) $_3C_6H_2$, NH_2 ;

R^2 is H, CN, COMe, $COOC_{1-4}alkyl$, $COOC_2H_4OMe$, $COOC_2H_4OPr(n)$;

10 R^3 is $C_6H_4R^6$, 3,4-OCH $_2$ O- C_6H_3 ; 2-furanyl;

R^6 is 4-Cl, 4-NO $_2$, 4-N($C_{1-4}alkyl$) $_2$, 3-($C_{1-4}alkyloxy$), 4-($C_{1-4}alkyloxy$),
3,4-($C_{1-4}alkyloxy$) $_2$, 3,4,5-($C_{1-4}alkyloxy$) $_3$;

R^4 is NH_2 , NHCOMe;

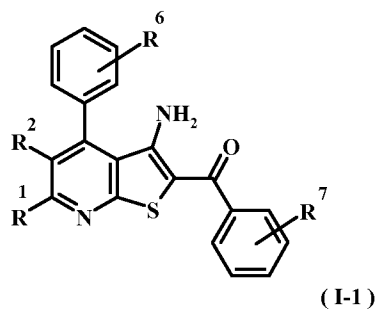
R^5 is CN, COMe, $COC_6H_4R^7$; CO-(2-naphthyl);

15 R^7 is H, 4-F, 4-Cl, 3-OMe, 4-OMe, 2,4-(OMe) $_2$, 3,4,5-(OMe) $_3$

and pharmaceutically acceptable salts, hydrates, solvates and polymorphs thereof.

2. Compounds according to claim 1 for use as multidrug resistance (MDR) modulators to increase the effectiveness of chemotherapy in cancer treatment.

3. Compounds of Claim 1 of the general formula (I-1),



wherein

R^1 is Me, 3,4,5-(OMe) $_3C_6H_2$;

R^2 is COMe, $COOC_{1-4}alkyl$, $COOC_2H_4OMe$, $COOC_2H_4OPr(n)$;

R^6 is 4-NMe $_2$, 3-OMe, 4-($C_{1-4}alkyloxy$), 3,4-(OMe) $_2$, 3,4,5-(OMe) $_3$;

25 R^7 is H, 4-F, 4-Cl, 3-OMe, 4-OMe, 2,4-(OMe) $_2$, 3,4,5-(OMe) $_3$

and pharmaceutically acceptable salts, hydrates, solvates and polymorphs thereof;

with the proviso that compounds of Formula I-1 do not include:

- 5-Acetyl-3-amino-2-benzoyl-6-methyl-4-(4-methoxyphenyl)thieno[2,3-*b*]pyridine,
3-Amino-2-benzoyl-6-methyl-4-phenylthieno[2,3-*b*]pyridine-5-carboxylic acid ethyl ester,
3-Amino-2-benzoyl-6-methyl-4-(4-methoxyphenyl)thieno[2,3-*b*]pyridine-5-carboxylic acid
5 ethyl ester,
3-Amino-2-benzoyl-4-(4-dimethylaminophenyl)-6-methylthieno[2,3-*b*]pyridine-
5-carboxylic acid ethyl ester,
3-Amino-2-benzoyl-4-(3,4-dimethoxyphenyl)-6-methylthieno[2,3-*b*]pyridine-
5-carboxylic acid ethyl ester,
10 3-Amino-4-benzo[1,3]dioxol-5-yl-2-benzoyl-6-methylthieno[2,3-*b*]pyridine-
5-carboxylic acid ethyl ester,
3-Amino-2-benzoyl-6-methyl-4-(3,4,5-trimethoxyphenyl)thieno[2,3-*b*]pyridine-
5-carboxylic acid ethyl ester,
3-Amino-2-(4-chlorobenzoyl)-4-(4-dimethylaminophenyl)-6-phenylthieno[2,3-*b*]pyridine,
15 3-Amino-4-(4-methoxyphenyl)-2-(4-methoxyphenylcarbamoyl)-6-methyl-
thieno[2,3-*b*]pyridine-5-carboxylic acid ethyl ester,
3-Amino-4-(4-bromophenyl)-2-(4-chlorobenzoyl)-6-methylthieno[2,3-*b*]pyridine-
5-carboxylic acid ethyl ester,
3-Amino-2-(4-chlorobenzoyl)-4-(4-dimethylaminophenyl)-6-methylthieno[2,3-*b*]pyridine-5-
20 carboxylic acid ethyl ester,
3-Amino-2-(4-chlorobenzoyl)-4-(4-chlorophenyl)-6-methylthieno[2,3-*b*]pyridine-
5-carboxylic acid ethyl ester,
3-Amino-2-(4-chlorobenzoyl)-4-furan-2-yl-6-methylthieno[2,3-*b*]pyridine-5-carboxylic acid
ethyl ester.
25
4. Compounds according to claim 3 for use as multidrug resistance modulators to increase
the effectiveness of chemotherapy in cancer treatment.
5. Compounds according to claim 3, selected from the group, containing:
- 30 3-Amino-2-(2,4-dimethoxybenzoyl)-6-methyl-4-(3,4,5-trimethoxyphenyl)thieno[2,3-
b]pyridine-5-carboxylic acid ethyl ester,
3-Amino-2-(4-methoxybenzoyl)-6-methyl-4-(3,4,5-trimethoxyphenyl)thieno[2,3-*b*]-
pyridine-5-carboxylic acid ethyl ester,
3-Amino-4-(3,4-dimethoxyphenyl)-2-(4-methoxybenzoyl)-6-methylthieno[2,3-*b*]-

pyridine-5-carboxylic acid methyl ester,

3-Amino-2-(4-fluorobenzoyl)-4-(3,4,5-trimethoxyphenyl)-6-methylthieno[2,3-*b*]

pyridine-5-carboxylic acid ethyl ester,

3-Amino-2-(2,4-dimethoxybenzoyl)-6-methyl-4-(3,4,5-trimethoxyphenyl)thieno[2,3-*b*]-

5 pyridine-5-carboxylic acid methyl ester,

3-Amino-2-(4-methoxybenzoyl)-6-methyl-4-(3,4,5-trimethoxyphenyl)thieno[2,3-*b*]-

pyridine-5-carboxylic acid methyl ester,

5-Acetyl-3-amino-2-(4-methoxybenzoyl)-6-methyl-4-(3,4,5-trimethoxyphenyl)thieno[2,3-*b*]pyridine,

10 3-Amino-2-(4-methoxybenzoyl)-6-methyl-4-(3,4,5-trimethoxyphenyl)thieno[2,3-*b*]-

pyridine-5-carboxylic acid 2-methoxyethyl ester,

3-Amino-2-(2,4-dimethoxybenzoyl)-6-methyl-4-(3,4,5-trimethoxyphenyl)thieno[2,3-*b*]-

pyridine-5-carboxylic acid 2-methoxyethyl ester,

3-Amino-2-benzoyl-6-methyl-4-(3,4,5-trimethoxyphenyl)thieno[2,3-*b*]pyridine-

15 5-carboxylic acid 2-methoxyethyl ester and

3-Amino-4-(4-hydroxy-3-methoxyphenyl)-2-(4-methoxybenzoyl)-6-methylthieno[2,3-*b*]-

pyridine-5-carboxylic acid ethyl ester

for use as multidrug resistance modulators to increase the effectiveness of chemotherapy in cancer treatment.

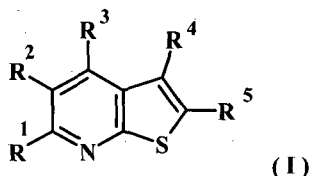
20

6. A pharmaceutical composition comprising as active ingredient a compound as claimed in any preceding claim, together with one or more pharmaceutically acceptable excipients or vehicles.

AMENDED CLAIMS

received by the International Bureau on 17 January 2013

1. Compounds of the general formula (I),



wherein

 R^1 is Me; C_6H_5 ; 3,4,5-(OMe) $_3C_6H_2$; NH_2 ; R^2 is H; CN; COMe; $COOC_{1-4}alkyl$; $COOC_2H_4OMe$; $COOC_2H_4OPr(n)$;10. R^3 is $C_6H_4R^6$; 3,4-OCH $_2$ O- C_6H_3 ; 2-furanyl; R^6 is 4-Cl; 4-NO $_2$; 4-OH; 3-OMe, 4-OH; 4-N($C_{1-4}alkyl$) $_2$; 3-($C_{1-4}alkyloxy$);4-($C_{1-4}alkyloxy$); 3,4-($C_{1-4}alkyloxy$) $_2$; 3,4,5-($C_{1-4}alkyloxy$) $_3$; R^4 is NH_2 ; NHCOMe; R^5 is CN; COMe; $COC_6H_4R^7$; CO-(2-naphthyl);15. R^7 is H; 4-F; 4-Cl; 3-OMe; 4-OMe; 2,4-(OMe) $_2$; 3,4,5-(OMe) $_3$

and pharmaceutically acceptable salts, hydrates, solvates and polymorphs thereof, with the proviso that compounds of Formula I do not include:

3-Acetylamino-6-amino-2-cyano-4-ethoxyphenylthieno[2,3-*b*]pyridine-5-carbonitrile;20. 2-Acetyl-3-amino-4-benzo[1,3]dioxol-5-yl-6-methylthieno[2,3-*b*]pyridine-5-carboxylic acid ethyl ester;5-Acetyl-3-amino-2-benzoyl-4-benzo[1,3]dioxol-5-yl-6-methylthieno[2,3-*b*]pyridine;5-Acetyl-3-amino-2-benzoyl-4-(4-dimethoxyphenyl)-6-methyl-thieno[2,3-*b*]pyridine;5-Acetyl-3-amino-2-benzoyl-4-(4-dimethylaminophenyl)-6-methyl-thieno[2,3-*b*]pyridine;5-Acetyl-3-amino-2-benzoyl-4-(2-furanyl)-6-methylthieno[2,3-*b*]pyridine;25. 5-Acetyl-3-amino-2-benzoyl-4-(4-methoxyphenyl)-6-methyl-thieno[2,3-*b*]pyridine;5-Acetyl-3-amino-2-(4-chlorobenzoyl)-4-(4-chlorophenyl)-6-methylthieno[2,3-*b*]pyridine;2-Acetyl-3-amino-4(4-chlorophenyl)-6methylthieno[2,3-*b*]pyridine;5-Acetyl-3-amino-4-(4-chlorophenyl)-2-cyano-6-methylthieno[2,3-*b*]pyridine;30. 2-Acetyl-3-amino-4-(4-chlorophenyl)-6-methylthieno[2,3-*b*]pyridine-5-carboxylic acid ethyl ester;2-Acetyl-3-amino-4-(4-chlorophenyl)-6-phenylthieno[2,3-*b*]pyridine;

- 5-Acetyl-3-amino-2-cyano-4-(3,4-dimethoxyphenyl)-6-methyl-thieno[2,3-*b*]pyridine;
5-Acetyl-3-amino-2-cyano-4-benzo[1,3]dioxol-5-yl-6-methylthieno[2,3-*b*]pyridine;
5-Acetyl-3-amino-2-cyano-4-(4-dimethylaminophenyl)-6-methyl-thieno[2,3-*b*]pyridine;
5-Acetyl-3-amino-2-cyano-4-(4-methoxyphenyl)-6-methyl-thieno[2,3-*b*]pyridine;
5 5-Acetyl-3-amino-2-cyano-4-(3-methoxyphenyl)-6-methyl-thieno[2,3-*b*]pyridine;
5-Acetyl-3-amino-2-cyano-6-methyl-4-(4-nitrophenyl)thieno[2,3-*b*]pyridine;
2-Acetyl-3-amino-4-(3,4-dimethoxyphenyl)-6-methylthieno[2,3-*b*]pyridine-5-carboxylic
acid ethyl ester;
2-Acetyl-3-amino-4-(4-dimethylaminophenyl)-6-methyl-thieno[2,3-*b*]pyridine;
10 2-Acetyl-3-amino-4-(4-dimethylaminophenyl)-6-methylthieno[2,3-*b*]pyridine-5-carboxylic
acid ethyl ester;
2-Acetyl-3-amino-4-(4-dimethylaminophenyl)-6-phenylthieno[2,3-*b*]pyridine;
2-Acetyl-3-amino-4-(2-furanyl)-6-methylthieno[2,3-*b*]pyridine;
2-Acetyl-3-amino-4-(2-furanyl)-6-methylthieno[2,3-*b*]pyridine-5-carboxylic acid ethyl ester;
15 2-Acetyl-3-amino-4-(2-furanyl)-6-phenyl-thieno[2,3-*b*]pyridine;
2-Acetyl-3-amino-4-(4-methoxyphenyl)-6-phenylthieno[2,3-*b*]pyridine-5-carboxylic acid
ethyl ester;
5-Acetyl-3-amino-6-methyl-4-(4-nitrophenyl)-2-phenylthieno[2,3-*b*]pyridine;
2-Acetyl-3-amino-6-phenyl-4-(3,4,5-trimethoxyphenyl)-thieno[2,3-*b*]pyridine;
20 2-Acetyl-3,6-diamino-4-(4-chlorophenyl)-thieno[2,3-*b*]pyridine-5-carbonitrile;
2-Acetyl-3,6-diamino-4-(4-methoxyphenyl)-thieno[2,3-*b*]pyridine-5-carbonitrile;
3-Amino-2,5-diacetyl-4-benzo[1,3]dioxol-5-yl-6-methylthieno[2,3-*b*]pyridine;
3-Amino-2,5-diacetyl-4-(2-furanyl)-6-methylthieno[2,3-*b*]pyridine;
3-Amino-2,5-diacetyl-4-(4-methoxyphenyl)-6-methyl-thieno[2,3-*b*]pyridine;
25 3-Amino-2,5-diacetyl-4-(4-methoxyphenyl)-6-methyl-thieno[2,3-*b*]pyridine;
3-Amino-4-benzo[1,3]dioxol-5-yl-2-benzoyl-6-methylthieno[2,3-*b*]pyridine-5-carboxylic
acid ethyl ester;
3-Amino-4-benzo[1,3]dioxol-5-yl-2-cyano-6-methylthieno[2,3-*b*]pyridine-5-carboxylic acid
ethyl ester;
30 3-Amino-2-benzoyl-4-(3,4-dimethoxyphenyl)-6-methylthieno[2,3-*b*]pyridine-5-carboxylic
acid ethyl ester;
3-Amino-2-benzoyl-4-(4-chlorophenyl)-6-methylthieno[2,3-*b*]pyridine;
3-Amino-2-benzoyl-4-(4-chlorophenyl)-6-phenylthieno[2,3-*b*]pyridine;

- 3-Amino-2-benzoyl-4-(4-chlorophenyl)-6-phenylthieno[2,3-*b*]pyridine-5-carboxylic acid ethyl ester;
- 3-Amino-2-benzoyl-4-(4-dimethylaminophenyl)-6-methyl-thieno[2,3-*b*]pyridine;
- 3-Amino-2-benzoyl-4-(4-dimethylaminophenyl)-6-methylthieno[2,3-*b*]pyridine-5-carboxylic acid ethyl ester;
- 5 3-Amino-2-benzoyl-4-(2-furanyl)-6-methylthieno[2,3-*b*]pyridine;
- 3-Amino-2-benzoyl-4-(2-furanyl)-6-methyl-thieno[2,3-*b*]pyridine-5-carboxylic acid ethyl ester;
- 3-Amino-2-benzoyl-4-(2-furanyl)-6-phenyl-thieno[2,3-*b*]pyridine;
- 10 3-Amino-2-benzoyl-4-(4-methoxyphenyl)-6-methylthieno[2,3-*b*]pyridine-5-carboxylic acid ethyl ester;
- 3-Amino-2-benzoyl-4-(3-methoxyphenyl)-6-phenylthieno[2,3-*b*]pyridine;
- 3-Amino-2-benzoyl-4-(4-methoxyphenyl)-6-phenylthieno[2,3-*b*]pyridine-5-carboxylic acid ethyl ester;
- 15 3-Amino-2-benzoyl-6-methyl-4-(3,4,5-trimethoxyphenyl)thieno[2,3-*b*]pyridine-5-carboxylic acid ethyl ester;
- 3-Amino-2-benzoyl-6-phenyl-4-(3,4,5-trimethoxyphenyl)-thieno[2,3-*b*]pyridine;
- 3-Amino-2-(4-chlorobenzoyl)-4-(4-chlorophenyl)-6-methylthieno[2,3-*b*]pyridine;
- 3-Amino-2-(4-chlorobenzoyl)-4-(4-chlorophenyl)-6-methylthieno[2,3-*b*]pyridine-5-
- 20 carboxylic acid ethyl ester;
- 3-Amino-2-(4-chlorobenzoyl)-4-(4-chlorophenyl)-6-phenylthieno[2,3-*b*]pyridine;
- 3-Amino-2-(4-chlorobenzoyl)-4-(4-chlorophenyl)-6-phenylthieno[2,3-*b*]pyridine-5-carboxylic acid ethyl ester;
- 3-Amino-2-(4-chlorobenzoyl)-4-(4-dimethylaminophenyl)-6-methyl-thieno[2,3-*b*]pyridine;
- 25 3-Amino-2-(4-chlorobenzoyl)-4-(4-dimethylaminophenyl)-6-methylthieno[2,3-*b*]pyridine-5-carboxylic acid ethyl ester;
- 3-Amino-2-(4-chlorobenzoyl)-4-(4-dimethylaminophenyl)-6-methylthieno[2,3-*b*]pyridine-5-carboxylic acid ethyl ester;
- 3-Amino-2-(4-chlorobenzoyl)-4-(4-dimethylaminophenyl)-6-phenylthieno[2,3-*b*]pyridine;
- 30 3-Amino-2-(4-chlorobenzoyl)-4-(4-dimethylaminophenyl)-6-phenylthieno[2,3-*b*]pyridine;
- 3-Amino-2-(4-chlorobenzoyl)-4-(2-furanyl)-6-methylthieno[2,3-*b*]pyridine-5-carboxylic acid ethyl ester;
- 3-Amino-2-(4-chlorobenzoyl)-4-(2-furanyl)-6-phenyl-thieno[2,3-*b*]pyridine;

- 3-Amino-2-(4-chlorobenzoyl)-4-(4-methoxyphenyl)-6-phenylthieno[2,3-*b*]pyridine-5-carboxylic acid ethyl;
- 3-Amino-2-(4-chlorobenzoyl)-4-(4-methoxyphenyl)-6-phenylthieno[2,3-*b*]pyridine-5-carboxylic acid ethyl ester;
- 5 3-Amino-2-(4-chlorobenzoyl)-6-phenyl-4-(3,4,5-trimethoxyphenyl)-thieno[2,3-*b*]pyridine;
3-Amino-4-(4-chlorophenyl)-2,5-diacetyl-6-methylthieno[2,3-*b*]pyridine;
3-Amino-4-(4-chlorophenyl)-2-cyano-6-methylthieno[2,3-*b*]pyridine;
3-Amino-4-(4-chlorophenyl)-2-cyano-6-methylthieno[2,3-*b*]pyridine-5-carboxylic acid ethyl ester;
- 10 3-Amino-4-(4-chlorophenyl)-2-(4-fluorobenzoyl)-6-methylthieno[2,3-*b*]pyridine;
3-Amino-4-(4-chlorophenyl)-2-(4-fluorobenzoyl)-6-phenylthieno[2,3-*b*]pyridine;
3-Amino-4-(4-chlorophenyl)-2-(4-methoxybenzoyl)-6-methylthieno[2,3-*b*]pyridine;
3-Amino-4-(4-chlorophenyl)-2-(3-methoxybenzoyl)-6-methylthieno[2,3-*b*]pyridine;
3-Amino-4-(4-chlorophenyl)-2-(4-methoxybenzoyl)-6-phenylthieno[2,3-*b*]pyridine;
- 15 3-Amino-4-(4-chlorophenyl)-2-(3-methoxybenzoyl)-6-phenylthieno[2,3-*b*]pyridine;
3-Amino-2-cyano-4-(3,4-dimethoxyphenyl)-6-methylthieno[2,3-*b*]pyridine-5-carboxylic acid ethyl ester;
3-Amino-2-cyano-4-(4-chlorophenyl)-6-phenylthieno[2,3-*b*]pyridine;
3-Amino-2-cyano-4-(4-dimethylaminophenyl)-6-methylthieno[2,3-*b*]pyridine;
- 20 3-Amino-2-cyano-4-(2-furanyl)-6-methylthieno[2,3-*b*]pyridine;
3-Amino-2-cyano-4-(2-furanyl)-6-phenylthieno[2,3-*b*]pyridine;
3-Amino-2-cyano-4-(4-methoxyphenyl)-6-methylthieno[2,3-*b*]pyridine-5-carboxylic acid ethyl ester;
3-Amino-2-(4-fluorobenzoyl)-4-(2-furanyl)-6-phenylthieno[2,3-*b*]pyridine;
- 25 3-Amino-2-(4-fluorobenzoyl)-6-phenyl-4-(3,4,5-trimethoxyphenyl)-thieno[2,3-*b*]pyridine;
3-Amino-2-(4-methoxybenzoyl)-6-phenyl-4-(3,4,5-trimethoxyphenyl)-thieno[2,3-*b*]pyridine;
3-Amino-2-naphthalenyl-6-phenyl-4-(3,4,5-trimethoxyphenyl)-thieno[2,3-*b*]pyridine;
2-Cyano-3,6-diamino-3,4,5-trimethoxyphenyl-thieno[2,3-*b*]pyridine-5-carbonitrile;
2-Cyano-3,6-diamino-4-butoxyphenyl-thieno[2,3-*b*]pyridine-5-carbonitrile;
- 30 2-Cyano-3,6-diamino-4-chlorophenyl-thieno[2,3-*b*]pyridine-5-carbonitrile;
2-Cyano-3,6-diamino-4-ethoxyphenyl-thieno[2,3-*b*]pyridine-5-carbonitrile;
2-Cyano-3,6-diamino-4-(2-furanyl)-thieno[2,3-*b*]pyridine-5-carbonitrile;
2-Cyano-3,6-diamino-4-methoxyphenyl-thieno[2,3-*b*]pyridine-5-carbonitrile;
3,6-Diamino-2-(3,4-dimethoxybenzoyl)-4-(2-furanyl)-thieno[2,3-*b*]pyridine-5-carbonitrile;

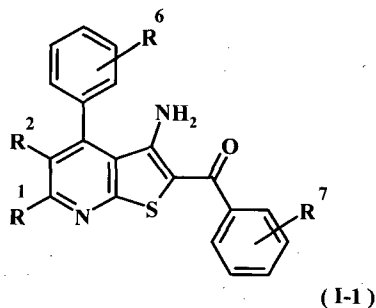
- 3,6-Diamino-2-(3,4-dimethoxybenzoyl)-4-(4-methoxyphenyl)-thieno[2,3-*b*]pyridine-5-carbonitrile;
- 3,6-Diamino-4-(3,4-dimethoxyphenyl)-2-(4-methoxybenzoyl)-thieno[2,3-*b*]pyridine-5-carbonitrile;
- 5 3,6-Diamino-2-benzoyl-4-(3,4,5-trimethoxyphenyl)-thieno[2,3-*b*]pyridine-5-carbonitrile;
- 3,6-Diamino-2-benzoyl-4-(3,4-dimethoxyphenyl)-thieno[2,3-*b*]pyridine-5-carbonitrile;
- 3,6-Diamino-2-benzoyl-4-(4-chlorophenyl)-thieno[2,3-*b*]pyridine-5-carbonitrile;
- 3,6-Diamino-2-benzoyl-4-(4-ethoxyphenyl)-thieno[2,3-*b*]pyridine-5-carbonitrile;
- 3,6-Diamino-2-benzoyl-4-(2-furanyl)-thieno[2,3-*b*]pyridine-5-carbonitrile;
- 10 3,6-Diamino-2-benzoyl-4-(4-methoxyphenyl)-thieno[2,3-*b*]pyridine-5-carbonitrile;
- 3,6-Diamino-2-(4-chlorobenzoyl)-4-(3,4,5-trimethoxyphenyl)-thieno[2,3-*b*]pyridine-5-carbonitrile;
- 3,6-Diamino-2-(4-chlorobenzoyl)-4-(3,4-dimethoxyphenyl)-thieno[2,3-*b*]pyridine-5-carbonitrile;
- 15 3,6-Diamino-2-(4-chlorobenzoyl)-4-(4-chlorophenyl)-thieno[2,3-*b*]pyridine-5-carbonitrile;
- 3,6-Diamino-2-(4-chlorobenzoyl)-4-(4-ethoxyphenyl)-thieno[2,3-*b*]pyridine-5-carbonitrile;
- 3,6-Diamino-2-(4-chlorobenzoyl)-4-(2-furanyl)-thieno[2,3-*b*]pyridine-5-carbonitrile;
- 3,6-Diamino-2-(4-chlorobenzoyl)-4-(4-methoxyphenyl)-thieno[2,3-*b*]pyridine-5-carbonitrile;
- 3,6-Diamino-2-(4-chlorophenyl)-4-(3,4-dimethoxybenzoyl)-thieno[2,3-*b*]pyridine-5-
- 20 carbonitrile;
- 3,6-Diamino-4-(4-chlorophenyl)-2-(4-fluorobenzoyl)-thieno[2,3-*b*]pyridine-5-carbonitrile;
- 3,6-Diamino-4-(4-chlorophenyl)-2-(4-methoxybenzoyl)-thieno[2,3-*b*]pyridine-5-carbonitrile;
- 3,6-Diamino-4-(4-ethoxyphenyl)-2-(4-methoxybenzoyl)-thieno[2,3-*b*]pyridine-5-carbonitrile;
- 25 3,6-Diamino-2-(4-fluorobenzoyl)-4-(3,4,5-trimethoxyphenyl)-thieno[2,3-*b*]pyridine-5-carbonitrile;
- 3,6-Diamino-2-(4-fluorobenzoyl)-4-(2-furanyl)-thieno[2,3-*b*]pyridine-5-carbonitrile;
- 3,6-Diamino-2-(4-fluorobenzoyl)-4-(4-methoxyphenyl)-thieno[2,3-*b*]pyridine-5-carbonitrile;
- 3,6-Diamino-4-(2-furanyl)-2-(4-methoxybenzoyl)-thieno[2,3-*b*]pyridine-5-carbonitrile;
- 30 3,6-Diamino-4-(2-furanyl)-2-(2-naphthalenylcarbonyl)thieno[2,3-*b*]pyridine-5-carbonitrile;
- 3,6-Diamino-2-(4-methoxybenzoyl)-4-(3,4,5-trimethoxyphenyl)-thieno[2,3-*b*]pyridine-5-carbonitrile;
- 3,6-Diamino-2-(4-methoxybenzoyl)-4-(4-methoxyphenyl)-thieno[2,3-*b*]pyridine-5-carbonitrile;

3,6-Diamino-2-naphthyl-4-(3,4,5-trimethoxyphenyl)-thieno[2,3-*b*]pyridine-5-carbonitrile.

2. Compounds according to claim 1 for use as P-gp, MRP1 and BCRP1 inhibitors to prevent multidrug resistance to increase the effectiveness of chemotherapy in cancer treatment.

5

3. Compounds of Claim 1 of the general formula (I-1),



wherein

R¹ is Me; 3,4,5-(OMe)₃C₆H₂;

10 R² is COMe; COOC₁₋₄alkyl; COOC₂H₄OMe; COOC₂H₄OPr(*n*);

R⁶ is 4-NMe₂; 3-OMe; 4-(C₁₋₄alkyloxy); 4-OH; 3-OMe, 4-OH; 3,4-(OMe)₂; 3,4,5-(OMe)₃;

R⁷ is H; 4-F; 4-Cl; 3-OMe; 4-OMe; 2,4-(OMe)₂; 3,4,5-(OMe)₃

and pharmaceutically acceptable salts, hydrates, solvates and polymorphs thereof;

with the proviso that compounds of Formula I-1 do not include:

15 5-Acetyl-3-amino-2-benzoyl-4-(4-dimethoxyphenyl)-6-methyl-thieno[2,3-*b*]pyridine;

5-Acetyl-3-amino-2-benzoyl-4-(4-dimethylaminophenyl)-6-methyl-thieno[2,3-*b*]pyridine;

5-Acetyl-3-amino-2-benzoyl-4-(4-methoxyphenyl)-6-methyl-thieno[2,3-*b*]pyridine;

5-Acetyl-3-amino-6-methyl-4-(4-nitrophenyl)-2-phenylthieno[2,3-*b*]pyridine;

3-Amino-2-benzoyl-4-(3,4-dimethoxyphenyl)-6-methylthieno[2,3-*b*]pyridine-5-carboxylic

20 acid ethyl ester;

3-Amino-2-benzoyl-4-(4-dimethylaminophenyl)-6-methylthieno[2,3-*b*]pyridine-5-carboxylic acid ethyl ester;

3-Amino-2-benzoyl-4-(4-methoxyphenyl)-6-methylthieno[2,3-*b*]pyridine-5-carboxylic acid ethyl ester;

25 3-Amino-2-benzoyl-6-methyl-4-(3,4,5-trimethoxyphenyl)thieno[2,3-*b*]pyridine-5-carboxylic acid ethyl ester;

3-Amino-2-(4-chlorobenzoyl)-4-(4-dimethylaminophenyl)-6-methylthieno[2,3-*b*]pyridine-5-carboxylic acid ethyl ester;

3-Amino-2-(4-chlorobenzoyl)-4-(4-dimethylaminophenyl)-6-methylthieno[2,3-*b*]pyridine-5-carboxylic acid ethyl ester

for use as multidrug resistance modulators to increase the effectiveness of chemotherapy in cancer treatment.

5 4. Compounds according to claim 3 for use as P-gp, MRP1 and BCRP1 inhibitors to prevent multidrug resistance and to increase the effectiveness of chemotherapy in cancer treatment.

5. Most preferred compounds according to claim 3

3-Amino-2-(2,4-dimethoxybenzoyl)-6-methyl-4-(3,4,5-trimethoxyphenyl)thieno[2,3-*b*]pyridine-5-carboxylic acid ethyl ester,

10 3-Amino-2-(4-methoxybenzoyl)-6-methyl-4-(3,4,5-trimethoxyphenyl)thieno[2,3-*b*]pyridine-5-carboxylic acid ethyl ester,

3-Amino-4-(3,4-dimethoxyphenyl)-2-(4-methoxybenzoyl)-6-methylthieno[2,3-*b*]pyridine-5-carboxylic acid methyl ester,

15 3-Amino-2-(4-fluorobenzoyl)-4-(3,4,5-trimethoxyphenyl)-6-methylthieno[2,3-*b*]pyridine-5-carboxylic acid ethyl ester,

3-Amino-2-(2,4-dimethoxybenzoyl)-6-methyl-4-(3,4,5-trimethoxyphenyl)thieno[2,3-*b*]pyridine-5-carboxylic acid methyl ester,

3-Amino-2-(4-methoxybenzoyl)-6-methyl-4-(3,4,5-trimethoxyphenyl)thieno[2,3-*b*]pyridine-5-carboxylic acid methyl ester,

20 5-Acetyl-3-amino-2-(4-methoxybenzoyl)-6-methyl-4-(3,4,5-trimethoxyphenyl)thieno[2,3-*b*]pyridine,

3-Amino-2-(4-methoxybenzoyl)-6-methyl-4-(3,4,5-trimethoxyphenyl)thieno[2,3-*b*]pyridine-5-carboxylic acid 2-methoxyethyl ester,

25 3-Amino-2-(2,4-dimethoxybenzoyl)-6-methyl-4-(3,4,5-trimethoxyphenyl)thieno[2,3-*b*]pyridine-5-carboxylic acid 2-methoxyethyl ester,

3-Amino-2-benzoyl-6-methyl-4-(3,4,5-trimethoxyphenyl)thieno[2,3-*b*]pyridine-5-carboxylic acid 2-methoxyethyl ester and

3-Amino-4-(4-hydroxy-3-methoxyphenyl)-2-(4-methoxybenzoyl)-6-methylthieno[2,3-*b*]pyridine-5-carboxylic acid ethyl ester

30 for use as multidrug resistance modulators to increase the effectiveness of chemotherapy in cancer treatment.

6. A pharmaceutical composition comprising as active ingredient a compound as claimed in any preceding claim, together with one or more pharmaceutically acceptable excipients or vehicles.

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2013/054767

A. CLASSIFICATION OF SUBJECT MATTER INV. C07D495/04 ADD. A61K31/4365		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) C07D		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, CHEM ABS Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GAD-ELKAREEM, MOHAMED ET AL: "Reactions with 3,6-diaminothieno[2,3-b]pyridines: synthesis and characterization of several new fused pyridine heterocycles", HETEROATOM CHEMISTRY, vol. 18, no. 4, 2007, pages 405-413, XP2714039, page 406; compounds 4a,b ----- -/--	1
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> See patent family annex.		
* Special categories of cited documents :		
"A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed		"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family
Date of the actual completion of the international search 2 October 2013		Date of mailing of the international search report 15/10/2013
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Menegaki, Fotini

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2013/054767

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ATTABY F A ET AL: "SYNTHESIS AND ANTIMICROBIAL EVALUATION OF SEVERAL NEW PYRIDINE, THIENOPYRIDINE AND PYRIDOTHIENOPYRAZOLE DERIVATIVES", PHOSPHORUS, SULFUR AND SILICON AND THE RELATED ELEMENTS, TAYLOR & FRANCIS INC, US, vol. 149, 1 January 1999 (1999-01-01), pages 49-64, XP008049443, ISSN: 1042-6507 page 59; compounds 6a,b -----	1
X	ATTABY F A: "REACTIONS OF STYRYLTHIENYL KETONE, STYRYL FURYL KETONE WITH THIOCYANOACETAMIDE: SYNTHESIS OF SEVERAL NEW PYRIDINES, THIENO-2,3-PYRIDINES, PYRIDO -2',3'-4,5-THIENO-3,2-PYRIDAZINES AND PYRIDO-3',2'-4,5THIENO-PYRIMIDIN-ONE DERIVATIVES", PHOSPHORUS, SULFUR AND SILICON AND THE RELATED ELEMENTS, TAYLOR & FRANCIS INC, US, vol. 139, no. 1, 1 August 1998 (1998-08-01), pages 1-12, XP008085437, ISSN: 1042-6507, DOI: 10.1080/10426509808035673 compounds 6b;20c,d -----	1
X	FAWZY ATTABY ET AL: "Reaction with cyanothioacetamide derivative: Synthesis and reactions of some thieno[2,3-b]pyridine, pyridothienopyridazine, pyrazolo[3,4-b]pyridine and pyridopyrazolo-1,2,4-triazine derivatives", PHOSPHORUS, SULFUR AND SILICON, vol. 1, 1996, pages 257-270, XP9173120, compounds 6b;10a,b -----	1
X	AZZA ABDEL-FATTAH ET AL: PHOSPHORUS, SULFUR AND SILICON, vol. 156, 1999, pages 53-68, XP9173121, page 60 - page 61; compounds 10b; 14a,b -----	1
X	EL-LOUH, ALI ET AL: "Synthesis of dihydropyridine, alkylthiopyridine, thienopyridine and pyridothienopyrimidine derivatives", ASIAN JOURNAL OF CHEMISTRY, vol. 19, no. 2, 2007, pages 1283-1292, XP9173114, page 1290; compounds 9-13 -----	1,3
	-/--	

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2013/054767

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SANAA ELDIN ET AL: "Cyanothioacetamide and its derivatives in heterocyclic synthesis: A new route for the synthesis of several pyridine and thieno[2,3-b]pyridine derivatives and their biological evaluation", Z.NATURFORSCHUNG, vol. 54b, 1998, pages 674-680, XP9173123, compounds 8a,b -----	1
X	GALAL ELGEMEIE ET AL: "a,b-unsaturated nitriles in heterocyclic synthesis. Novel synthesis of pyridines and thieno[2,3-b]pyridine derivatives", THE CHEMICAL SOCIETY OF JAPAN, vol. 61, 1988, pages 4431-4433, XP9173124, compounds 6a-h -----	1,3
A	EP 2 308 883 A1 (CENTRE NAT RECH SCIENT [FR]) 13 April 2011 (2011-04-13) examples -----	1-6
A	WO 2007/135592 A1 (UNIV HONG KONG POLYTECHNIC [CN]; CHAN TAK-HANG [CA]; CHOW LARRY MING-C) 29 November 2007 (2007-11-29) examples -----	1-6
A	WO 2004/111052 A1 (XENOVA LTD [GB]; WANG SHOUMING [GB]; HANCOX TIMOTHY [GB]; MILLER WARRE) 23 December 2004 (2004-12-23) examples -----	1-6
X	SHARANIN, YU. A.; KRIVOKOLYSKO, S. G.; DYACHENKO, V. D.: ZHURNAL ORGANICHESKOI KHIMII, vol. 30, no. 4, 1994, pages 581-587, XP002714041, ISSN: 0514-7492 page 582; compounds XIII b,c,n -----	1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2013/054767

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 2308883	A1	13-04-2011	EP 2308883 A1
			WO 2011042860 A2

WO 2007135592	A1	29-11-2007	AU 2007252947 A1
			CA 2652617 A1
			CN 101454305 A
			EP 2029568 A1
			JP 2009537630 A
			US 2009197943 A1
			WO 2007135592 A1

WO 2004111052	A1	23-12-2004	NONE
