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Design, synthesis and biological evaluation of new potent and highly selective ROS1-tyrosine kinase inhibitor

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ABSTRACT

ROS1 protein is a receptor tyrosine kinase that has been reported mainly in meningiomas and astrocytomas, and until now, there is no selective inhibitor for this kinase. In this study, we illustrate for the synthesis of a highly potent and selective inhibitor for ROS1 kinase. The synthesized compound **1** was tested initially at a single dose concentration of 10 μM over 45 different kinases. At this concentration, a 94% inhibition of the enzymatic activity of ROS1 kinase was observed, while the inhibition in activity was below 30% in all of the other kinases. The pyrazole compound **1** was further tested in a 10-dose IC_{50} mode and showed an IC_{50} value of 199 nM for ROS1 kinase. The compound **1** can be used as a promising lead for the development of new selective inhibitors for ROS1 kinase, and it may open the way for new selective therapeutics for astrocytomas.

Signal transduction is an essential biological process for normal cell growth and function. The transduction of many of these signals is mediated through growth factors which transmit their signals into the cell by a group of trans-membrane proteins with intrinsic tyrosine kinase activity, named receptor tyrosine kinases (RTKs).[1–4] Mutations at RTKs encoding genes are associated with the incidence of several types of cancers.[5–8] ROS1 is a human gene that encodes for a trans-membrane receptor tyrosine kinase.[9–11] It is located at the chromosome 6 region 6q16→6q22.[9] This region of chromosome 6 is involved in non-random chromosomal arrangements in specific neoplasias including acute lymphoblastic leukemias, malignant melanoma, and ovarian carcinoma.[9] A micro-deletion at 6q21 of ROS1 results in the fusion of a golgi apparatus-associated protein called FIG (Fused in Glioblastoma), to the kinase domain of the protooncogene ROS1 producing a chimeric protein with a constitutive receptor tyrosine kinase activity.[12,13]

This fused chimeric protein is a potent oncogene and its transforming potential lies in its ability to interact with golgi apparatus.[12]

The ectopic expression of ROS1 receptor protein has been reported mainly in meningiomas and astrocytomas (25% of low grade and 30% of malignant glioma tumors) suggesting a key role for ROS1 in these CNS malignancies.[12,14] A survey of 45 different human tumor cell lines, made by Birchmeier et al.,[11] has showed that ROS1 was expressed in glioblastoma-derived cell lines at high levels, while not expressed at all or expressed minimally in the remaining cell lines.

Glioblastoma multiforme is the most advanced astrocytic neoplasm, and is one of the most aggressive human cancers with a median survival of less than one year. The complete resection of glioblastoma is impossible because of the diffuse infiltration of tumor cells into normal parenchyma. In addition, these tumors are highly resistant to radiation and chemotherapy. Despite decades of therapeutic research, effective chemotherapeutic treatment for high grade astrocytomas is not yet available, and patient care ultimately focuses on palliative management.[15–18]

Herein, we report the discovery of a potent and highly selective ROS1 RTK inhibitor **1** (Fig. 1). The synthetic and screening protocols

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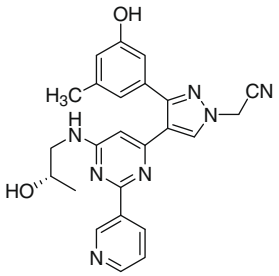
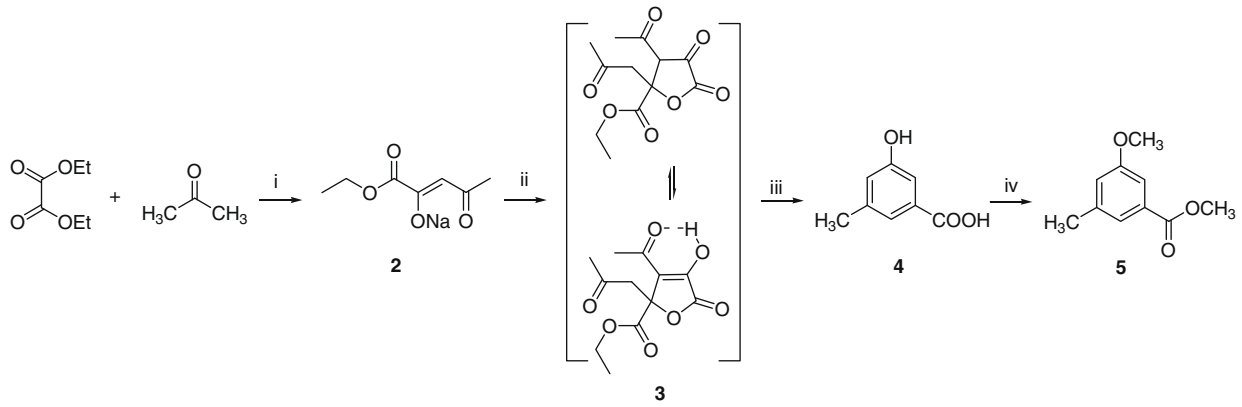


Figure 1. Structure of pyrazole compound **1**.

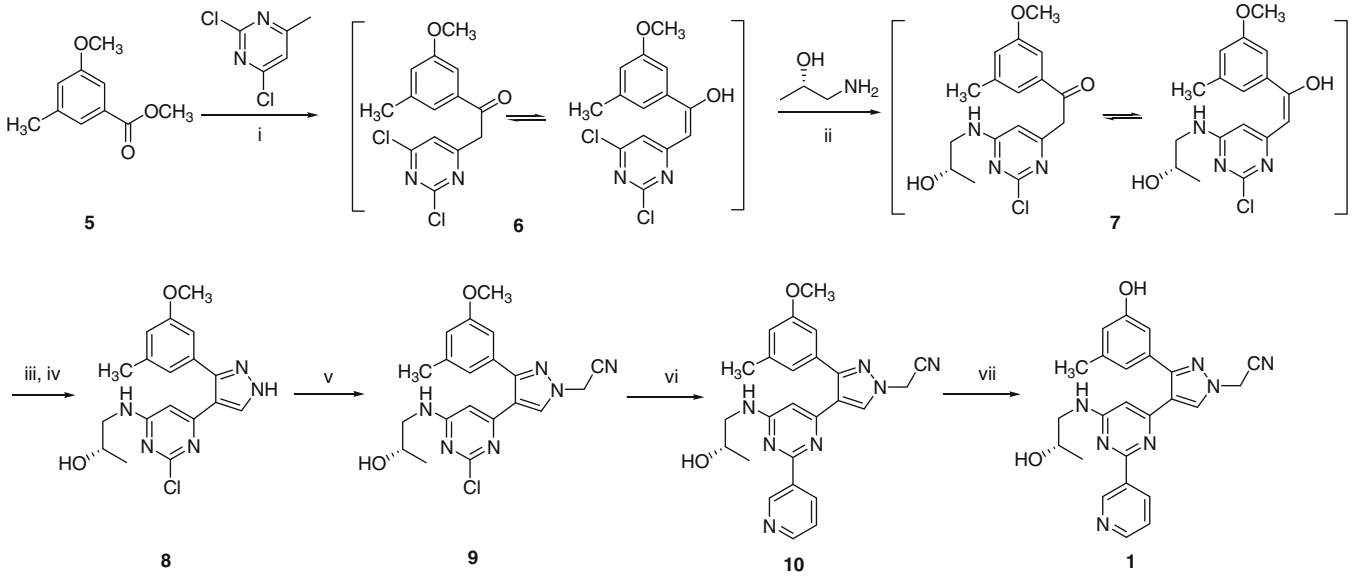
for the inhibitor are illustrated in details. The kinase inhibitory activity of the synthesized compound was tested over 45 different kinases, and it showed high selectivity for ROS1 kinase. According to our knowledge, this is the first selective ROS1 tyrosine kinase inhibitor.

For the synthesis of the target compound **1**, it was important at first to prepare the key ester, methyl 3-methoxy-5-methylbenzoate (**5**) as illustrated in Scheme 1. The synthesis started with the preparation of the sodium salt of ethyl 2-hydroxy-4-oxopent-2-enoate (**2**) according to the literature procedure,[19] through the condensation of diethyl oxalate with acetone in the presence of sodium ethoxide in absolute ethanol. The resulted salt **2** was then cyclized into Claisen furan derivative **3** by heating in 50% acetic acid followed by acidification with sulfuric acid.[20] The resulted Claisen compound underwent rearrangement and aromatization into 3-hydroxy-5-methylbenzoic acid (**4**) within less than 1 h by heating with magnesium oxide in boiling water, followed by acidification with hydrochloric acid to precipitate the product.[20] Methyl esterification and *O*-methylation of the resulted phenolic acid **4** were achieved in a single step and in high yield (94%) to give compound **5** through a little modification of the literature procedure,[21] where the acid **4** was refluxed with excess potassium carbonate and iodomethane in acetone in the presence of a catalytic amount of DMAP.

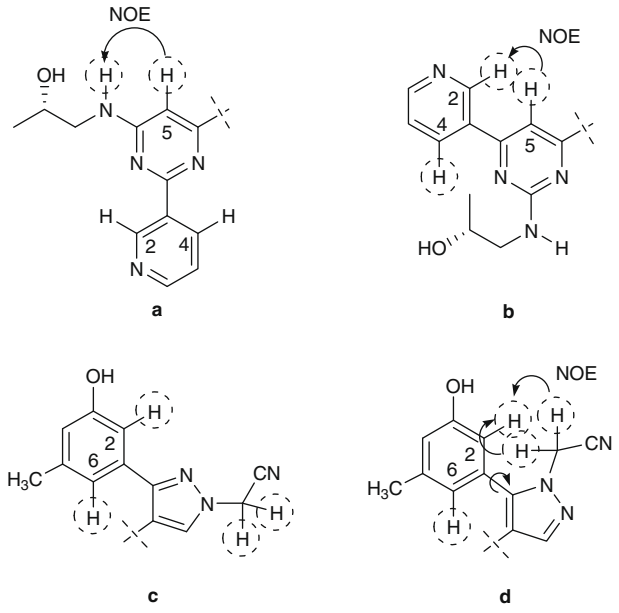
In Scheme 2, the benzoate ester **5** underwent a nucleophilic attack at its carboxylic carbon by the activated methylene group of 2,4-dichloro-6-methyl-pyrimidine. The activation of this methyl



Scheme 1. Reaction conditions and reagents: (i) NaOEt, abs. EtOH, rt, 4 h, 87%; (ii) acetic acid : H_2O (1:1), rt, 2 h, 50%; (iii) MgO, H_2O, reflux, 45 min, 42%; (iv) K_2CO_3, CH_3I, DMAP, acetone, 65 °C, 12 h, 94%.



Scheme 2. Reaction conditions and reagents: (i) LHMDS, THF, N_2, rt, 24 h, 50%; (ii) THF, 80 °C, 3 h, 35%; (iii) DMF–DMA, 90 °C, 20 h; (iv) hydrazine hydrate, abs. EtOH, rt, 12 h, 44%; (v) K_2CO_3, iodoacetonitrile, acetone, reflux, 2 h, 43%; (vi) 3-pyridineboronic acid, $Pd(PPh_3)_2Cl_2$, Na_2CO_3, N_2, CH_3CN/H_2O (1:1), 78 °C, 3 h, 67%; (vii) $BF_3{\cdot}S(CH_3)_2$, dichloromethane, N_2, rt, 12 h, 45%.



a: NOE effect between aminopropan-2-ol NH and Pyrimidine H_5 in compound **10**; **b**: Proposed NOE effect between pyrimidine H_5 and H_2 or H_4 of pyridine in compound **10** regioisomer; **c**: The distance between acetonitrile -CH_2- and the aromatic protons doesn't allow for NOE interaction in compound **10**; **d**: Proposed NOE effect between acetonitrile -CH_2- and the aromatic protons in the *2H*-pyrazole isomer of compound **10**.

Figure 2. NOE interactions in compound **10** and its proposed isomers.

group into an active methylene was achieved by dropwise addition of lithium bis(trimethylsilyl)amide (LHMDS) in dry THF at room temperature. The resulted tautomeric α,β-unsaturated ketone **6** was then subjected to a nucleophilic substitution reaction with the amino group of (*S*)-(+)-1-aminopropan-2-ol by heating at 80 °C in dry THF for 3 h. Two regioisomers were produced from this reaction, but the major isomer was (*S*)-2-(6-(2-hydroxypropylamino)-2-chloropyrimidin-4-yl)-1-(3-methoxy-5-methylphenyl)-ethanone (**7**), which was separated in a pure form using column chromatography. The structure of this isomer was confirmed by the 2D-NOESY NMR spectrum of the subsequent compound **10**. The conversion of the resulted tautomeric product **7** to the required pyrazole derivative **8** was achieved through two successive steps. In the first step, compound **7** was heated with excess *N*,*N*-dimethylformamide dimethylacetal for 20 h, and the resulted product was taken to the next step without further purification, where it was cyclized with hydrazine monohydrate in absolute ethanol into the pyrazole derivative **8**. The reaction of the resulted pyrazole **8** with iodoacetonitrile in the presence of excess potassium carbonate produced two different regioisomers. The intended isomer **9** was the major product of the reaction with R_f value of 0.54 (EtOAc) while the other isomer was produced as a minor product with higher R_f value of 0.66 (EtOAc). The required isomer **9** was separated in a pure form by column chromatography and its structure was confirmed by 2D-NOESY NMR spectrum of the subsequent compound **10**. Compound **10** was prepared in a moderate yield (67%) by Suzuki coupling of compound **9** with 3-pyridineboronic acid, in the presence of dichloro bis(triphenylphosphine)Pd(II) and sodium carbonate, in a mixed solvent of acetonitrile and water in a (1:1) ratio. The 2D-NOESY NMR spectrum of this compound confirmed the former nucleophilic substitution with (*S*)-(+)-1-aminopropan-2-ol in compound **7** at the 6-position of the pyrimidine ring (not the 2-position). This was proved by the absence of a cross peak between H_5 of the pyrimidine ring and H_2 or H_4 of the pyridyl group (Fig. 2a).

The presence of such cross peak would be expected if the substitution with (*S*)-(+)-1-aminopropan-2-ol occurred at the C_2 of the pyrimidine, since in this case, the subsequent substitution with pyridin-3-yl moiety would happen at the 6-position of pyrimidine, allowing for through space interaction between H_5 of the pyrimidine and H_2 or H_4 of the pyridine to occur (Fig. 2b). Furthermore, the presence of a cross peak between pyrimidine H_5 and the NH of the aminopropan-2-ol provides another evidence for the presence of the aminopropan-2-ol group at the 6-position of the pyrimidine ring (Fig. 2a). It was important also to prove that the former isomer **9** is the intended 1H-pyrazole not the 2H-pyrazole. This was proved too by the 2D-NOESY NMR spectrum of compound **10**, by the absence of any cross peak that might indicate a through space interaction between the –CH_2– protons of the acetonitrile group and any of the aromatic protons of the 3-methoxy-5-methylphenyl group (Fig. 2c). The presence of such cross-peaks would be expected if this isomer is the 2H-pyrazole, since the acetonitrile group would be close enough to the aromatic protons of 3-methoxy-

Table 1
Percentages of enzymatic inhibitions exerted by compound **1**[26] on 45 kinases[27]

Kinase enzyme	% Inhibition[a–d]
ABL1	20.44
AKT1 (dPH, S473D)	−2.97
Aurora A	21.75
BRAF	8.97
CDK1/cyclinB	3.64
CHK1	2.36
CK1epsilon	9.32
c-Kit	2.85
c-MET	5.82
c-Src	16.18
DAPK1	−1.17
DNA-PK	6.56
EGFR	−2.23
EPHA1	23.27
FAK/PTK2	3.43
FGFR1	−8.05
FGR	3.34
FLT1	−1.67
FYN	−7.86
HIPK1	−3.43
IKKa/CHUK	1.37
IR	7.01
JAK1	9.15
JNK1a1	3.83
KDR/VEGFR2	−5.84
LCK	10.28
LYN	−1.45
MEK1	−6.97
MST4	−1.54
MUSK	−0.42
P38a/MAPK14	5.27
p70S6K	5.79
PAK4	1.37
PIM1	5.91
PKCa	9.23
PLK1	5.85
RAF1	20.39
RET	6.65
ROCK1	11.72
RON/MST1R	28.82
ROS/ROS1	93.92
SYK	8.23
TIE2/TEK	8.17
TRKA/NTRK1	−0.14
YES	−2.59

[a] % Activity in each enzyme is the mean of two different readings.
[b] Test compound was used in a single dose concentration of 10 μM.
[c] 100% Activity refers to enzyme activity in negative control (DMSO).
[d] % Inhibition was calculated by subtracting % activity from 100.

5-methylphenyl group to exhibit NOE effect as shown in Figure 2d. The final hydroxyl product **1** was obtained by demethylation of the methoxy group of compound **10** using 10 equiv of borontrifluoride–dimethylsulfide complex in dichloromethane.

The screening results of the target compound **1** over 45 different kinases have revealed that the inhibitory activity of the compound was not exhibited over almost all of the tested kinases, while high potency and activity was selectively shown at ROS1 kinase only (Table 1).[22]

The compound was tested initially at a single dose concentration of 10 μM. At this concentration, 94% inhibition of the enzymatic activity of ROS1 kinase was observed, while the inhibition in activity was below 30% in all of the other kinases, and in the range of 20–30% in 5 kinases only (ABL1, Aurora A, EPHA1, RAF1 and RON/MST1R).

Compound **1** was further tested over ROS1 kinase in order to determine its IC_{50}, where a 10-dose IC_{50} mode with threefold serial dilutions starting at 20 μM concentration was applied against Staurosporine[23–25] as a reference standard. The compound has showed an IC_{50} value of 199 nM, while the IC_{50} value for the non-selective kinase inhibitor Staurosporine was 0.07 nM.

The high selectivity of compound **1** to ROS1 kinase and the diminished activity over the other kinases could be attributed to the increased bulkiness exerted by the substituents at pyrimidine nucleus. This increase in bulkiness seems to hinder the fitting of the compound to the binding sites of most of these kinases and to exclude it from their binding pockets. However, the selective inhibition of ROS1 kinase might be owed to a unique difference in the geometry of the binding pocket of this enzyme that enables the fitting and interaction of compound **1**. An indirect inhibitory effect of compound **1** at ROS1 kinase, probably through binding at an allosteric binding site, is another possible assumption for the reason behind this selective inhibition. The absolute reason is still unclear and we believe that it worth further exploration for the mechanism of its unique inhibitory effect.

In conclusion, a highly potent and selective inhibitor for ROS1 kinase has been synthesized and can be used as a promising lead for new selective inhibitors for ROS1 kinase. It worth also to mention that until now, no selective inhibitor is available for ROS1 kinase, and the development of new selective inhibitors for such kinase might open the way for new selective therapeutics for astrocytomas. Screening of compound **1** against glioblastoma-derived tumors is currently undergoing.

Acknowledgments

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References and notes

1. Alaoui-Jamali, M. A. *Biomed. Pharmacother.* **2006**, *60*, 629.
2. Chase, A.; Cross, N. C. P. *Clin. Sci.* **2006**, *111*, 233.
3. Perona, R. *Clin. Transl. Oncol.* **2006**, *8*, 77.
4. Hubbard, S. R.; Miller, W. T. *Curr. Opin. Cell Biol.* **2007**, *19*, 117.
5. Gupta, R.; Knight, C. L.; Bain, B. J. *Br. J. Haematol* **2002**, *117*, 489.
6. Chalandon, Y.; Schwaller, J. *Haematologica* **2005**, *90*, 949.
7. Lengyel, E.; Sawada, K.; Salgia, R. *Curr. Mol. Med.* **2007**, *7*, 77.
8. Sequist, L. V.; Lynch, T. J. *Annu. Rev. Med.* **2008**, *59*, 429.
9. Nagarajan, L.; Louie, E.; Tsujimoto, Y.; Balduzzi, P. C.; Huebner, K.; Croce, C. M. *Proc. Natl. Acad. Sci. U. S. A.* **1986**, *83*, 6568.
10. Matsushime, H.; Wang, L. H.; Shibuya, M. *Mol. Cell Biol.* **1986**, *6*, 3000.
11. Birchmeier, C.; Sharma, S.; Wigler, M. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 9270.
12. Charest, A.; Kheifets, V.; Park, J.; Lane, K.; McMahon, K.; Nutt, C. L.; Housman, D. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 916.
13. Charest, A.; Lane, K.; McMahon, K.; Park, J.; Preisinger, E.; Conroy, H.; Housman, D. *Gene. Chromosome Canc.* **2003**, *37*, 58.
14. Jun, H. J.; Woolfenden, S.; Coven, S.; Lane, K.; Bronson, R.; Housman, D.; Charest, A. *Cancer Res.* **2009**, *69*, 2180.
15. Holland, E. C. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 6242.
16. Hess, S. M.; Anderson, J. G.; Bierbach, U. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 443.
17. Van de Kelft, E. *Acta Neurochir.* **1997**, *139*, 589.
18. Ohgaki, H.; Kleihues, P. *Am. J. Pathol.* **2007**, *170*, 1445.
19. Wipf, P.; Mahler, S. G.; Okumura, K. *Org. Lett.* **2005**, *7*, 4483.
20. Turner, F. A.; Gearien, J. E. *J. Org. Chem.* **1959**, *24*, 1952.
21. De Frutos, O.; Atienza, C.; Echavarren, A. M. *Eur. J. Org. Chem.* **2001**, *1*, 163.
22. Karaman, M. W.; Herrgard, S.; Treiber, D. K.; Gallant, P.; Atteridge, C. E.; Campbell, B. T.; Chan, K. W.; Ciceri1, P.; Davis, M. I.; Edeen, P. T.; Faraoni1, R.; Floyd, M.; Hunt, J. P.; Lockhart, D. J.; Milanov, Z. V.; Morrison, M. J.; Pallares, G.; Patel, H. K.; Pritchard, S.; Wodicka, L. M.; Zarrinkar, P. P. *Nat. Biotechnol.* **2008**, *26*, 127.
23. Omura, S.; Sasaki, Y.; Iwai, Y.; Takeshima, H. *J. Antibiot. (Tokyo)* **1995**, *48*, 535.
24. Gescher, A. *Crit. Rev. Oncol. Hematol.* **2000**, *34*, 127.
25. Yang, S.; Malaviya, R.; Wilson, L. J.; Argentieri, R.; Chen, X.; Yang, C.; Wang, B.; Cavender, D.; Murray, W. V. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 326.
26. *Preparation of compound* **1**: To a solution of compound **10** (75 mg, 0.16 mmol) in dichloromethane (3 mL) was added borontrifluoride–methyl sulfide complex (172 μL, 1.65 mmol) in a dropwise manner at room temperature and under N_2 atmosphere. The resulting suspension was stirred for 12 h, and then the mixture was concentrated under vacuum. The residue was partitioned between ethylacetate (100 mL) and brine (50 mL). The organic layer was separated and dried over anhydrous $MgSO_4$, then evaporated under vacuum. The residue was purified by column chromatography (silica gel, methanol-dichloromethane 1:30, v/v) to yield the pure hydroxyl product **1** as a white powder (32.5 mg, 45%); mp 236–237 °C; ^{1}H NMR (CD_3OD) δ 1.09 (d, J = 5.7 Hz, 3H), 2.21 (s, 3H), 3.25 (s, 1H), 3.38 (br s, 1H), 3.87 (br s, 1H), 5.32 (s, 2H), 6.65 (s, 1H), 6.69 (s, 1H), 6.76 (s, 1H), 6.86 (s, 1H), 7.38–7.41 (m, 1H), 8.14 (d, J = 8.1 Hz, 1H), 8.28 (s, 1H), 8.49 (d, J = 3.5 Hz, 1H), 8.81 (s, 1H); IR (KBr) 3425, 2926, 2360, 1586, 1454, 1349, 1162 cm^{-1}.
27. Kinase assays were performed at Reaction Biology Corporation using the 'HotSpot' assay platform. Kinase assay protocol; reaction buffer: base reaction buffer; 20 mM Hepes (pH 7.5), 10 mM $MgCl_2$, 1 mM EGTA, 0.02% Brij35, 0.02 mg/ml BSA, 0.1 mM Na_3VO_4, 2 mM DTT, 1% DMSO. Required cofactors were added individually (if needed) to each kinase reaction. Reaction Procedure: To a freshly prepared buffer solution was added any required cofactor for the enzymatic reaction, followed by the addition of the selected kinase at a concentration of 20 μM. The contents were mixed gently, then the compound under test (compound **1**) dissolved in DMSO was added to the reaction mixture in the appropriate concentration. 339-ATP (specific activity 500 μCi/μL) was added to the mixture in order to initiate the reaction, and the mixture was incubated at room temperature for 2 h. Initial screening over 45 kinases: Compound **1** was tested by single dose duplicate made at a concentration of 10 μM. Staurosporine was used as a control compound in a 5-dose IC_{50} mode with 10-fold serial dilutions starting at 20 μM. Reaction was carried out at 10 μM ATP concentration. Testing against ROS1 kinase: compound **1** was tested in a 10-dose IC_{50} mode with threefold serial dilutions starting at 20 μM. Staurosporine was used as a control compound in a 10-dose IC_{50} mode with fivefold serial dilutions starting at 20 μM. Reaction was carried out at 10 μM ATP concentration.