2-Thiazolylimino/Heteroarylimino-5-arylidene-4-thiazolidinones as New Agents with SHP-2 Inhibitory Action

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SHP-2, a nonreceptor protein tyrosine phosphatase encoded by the PTPN11 gene, mediates cell signaling by growth factors and cytokines via the RAS/MAP kinase pathway. Somatic mutations in PTPN11 gene account for approximately 18% of juvenile myelomonocytic leukemia (JMML) patients. Moreover, SHP-2 mutations leading to continuously active enzyme were found in more than 50% of Noonan syndrome patients and are considered to be responsible for the high tendency of these patients to juvenile leukemias and other cancer types. Recently SHP-2 became a new drug target, but till now little has been done in this field. In the present study, 17 2-thiazolylimino/heteroarylimino-5-arylidene-4-thiazolidinones divided into three series of derivatives bearing thiazole-, benzo[*d*]thiazole-, and benzo[*d*]isothizole rings were tested for SHP-2 inhibitory activity. Most of the compounds were good SHP-2 inhibitors. Benzo[*d*]thiazole derivatives exhibited the best inhibitory action. Docking studies revealed that hydrophobic interactions and hydrogen bond formation stabilize enzyme−inhibitor complex.

Introduction

SHP-2 is a nonmembranic protein tyrosine phosphatase that mediates cell signaling by growth factors and cytokines acting via the RAS/MAP kinase pathway.[1] The enzyme is normally inactive because N-SH2 domain blocks the active site. It is activated when its SH2 domains are connected to other tyrosine phosphorylated proteins.[2] Mutations of somatic or germline origin in the PTPN11 gene,[3–5] encoding the enzyme, were found in many cases of leukemia and myeloblastic disorders as well as in Noonan syndrome, which is characterized by high tendency to juvenile myelomonocytic leukemia (JMML[a]). Although JMML[6] is the most common hematologic disorder in which PTPN11 mutations were observed, cases of acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic myelomonocytic leukemia (CMML), and myelodysplastic syndromes (MDS)[7] with PTPN11 mutations are mentioned.[3] Moreover, PTPN11 mutations were observed in patients suffering from Leopard syndrome (LS),[8,9] which is phenotypically related to Noonan syndrome (NS).

Noonan syndrome is a genetic disorder that causes abnormal development of multiple parts of the body, low stature, congenital heart diseases, especially pulmonary valve stenosis, and other disorders.[10] Moreover, NS patients often suffer from inflammations.[11]

PTPN11 mutations leading to continuously active SHP-2 were found in more than 50% of Noonan syndrome patients and are considered to be responsible for the high tendency of these patients to juvenile leukemias and other types of cancer. The 46% of the mutations observed in NS and relative disorders and 91% of the mutations present in hematologic disorders are located at the N-SH2 domain.[1]

Noonan syndrome affects 1 in 1000−2500 children,[12] while leukemia accounts for 2% of adult cancers and $^1/_3$ of childhood cancers.[13] Since SHP-2 overactivation, due to PTPN11 gene mutations, seems to be the cause of a great portion of these incidents, regulation of enzyme activity would prevent disease development in many patients. Specific SHP-2 inhibitors could be used for the treatment of individuals bearing these mutations.

PTPN11 gene was first connected to Noonan syndrome[4] and leukemia[7] incidences in 2001 and 2003, respectively. Thus, SHP-2 became a drug target only the past few years, and little has been done in this field till now. A number of SHP-2 inhibitors have been found accidentally during researchers' efforts to discover PTP1B or other tyrosine phosphatase inhibitors.[14–16] In 2006, Nören-Müller and co-workers, following biological oriented synthesis, designed and synthesized a number of furanodictin A derivatives with SHP-2 inhibitory action,[17] while Chen and co-workers discovered a novel potent SHP-2 inhibitor, 8-hydroxy-7-(2-(6-sulfonaphthalen-2-yl)hydrazinyl)quinoline-5-sulfonic acid (NSC-87877).[18] In the experimental conditions used by Nören-Müller and co-workers, the best furanodictin A derivative **2a**, (2*S*,3a*R*,6*S*,6a*R*)-*N*-(cyclohexylmethyl)-6-(5-((*R*)-3-(pyrrolidin-1-ylmethyl)phenyl)-1*H*-tetrazol-1-yl)hexahydrofuro[3,2-*b*]furan-2-amine (Figure 1), exhibited an IC_{50} value of 2.47 μM using *p*-nitrophenyl phosphate as substrate. NSC-87877 inhibitor, evaluated using 20 μM 6,8-difluoro-4-methylumbellferyl phosphate as substrate, exhibited a much lower IC_{50} value: 0.318 μM. NSC-87877 is the most potent SHP-2 inhibitor known till now, although a straight comparison with furanodictin A derivatives cannot be done, since different experimental conditions were used. The compound, bearing a naphthalene 1-sulfonate and a 8-hydroxyquinoline 5-sulfonate group connected via a −N=N− bridge, was considered to be stabilized in the active center cleft via hydrogen bond interactions between sulfonate groups and Arg465, Lys280, and Asn281.

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[a] Abbreviations: MAP, mitogen activated protein; PTPN 11, protein tyrosine phosphatase nonreceptor type 11; JMML, juvenile myelomonocytic leukemia; ALL, acute lymphoblastic leukemia; CMML, chronic myelomonocytic leukemia; MDS, myelodysplastic syndrome; LS, Leopard syndrome; NS, Noonan syndrome.

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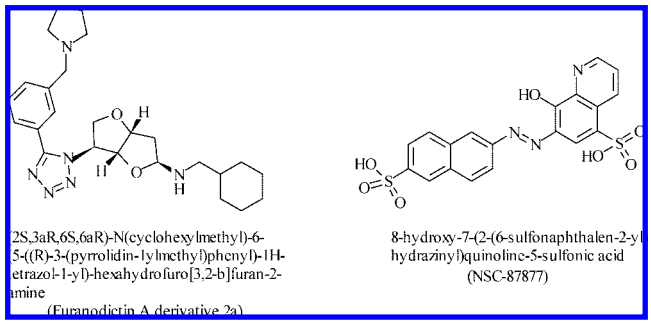


Figure 1. Structures of the SHP-2 inhibitors furanodictin A derivative **2a** and compound NSC-87877.

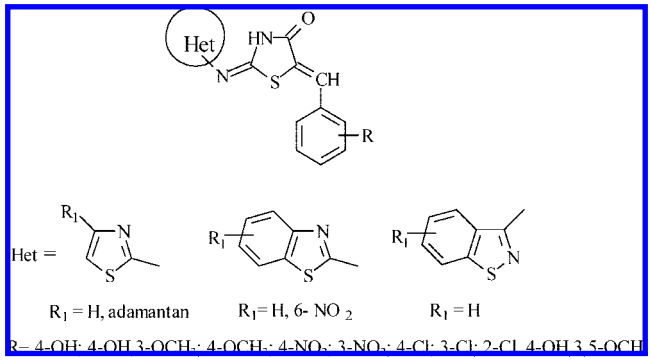


Figure 2. Structures of tested compounds.

During the past decade, many thiazole and thiazolidinone derivatives have been tested for protein phosphatase inhibitory action. Because of their structural resemblance to the natural peptide substrates of the enzymes, many derivatives of these series have been found to effectively inhibit protein phosphatases, mainly PTP1B.[19–27]

Taking into account previous knowledge in this field, we synthesized and tested a series of 2-thiazolylimino/heteroarylimino-5-arylidene-4-thiazolidinone derivatives for SHP-2 inhibitory activity. According to the heteroarylimino group, the compounds can be divided into three series bearing thiazole-, benzo[*d*]thiazole-, and benzo[*d*]isothiazole moieties. In order to analyze the mode of binding of different derivatives, docking studies for selected compounds were performed.

Results and Discussion

Chemistry. The compounds described in this paper (Figure 2) were synthesized by a three-step reaction protocol reported earlier by us.[28]

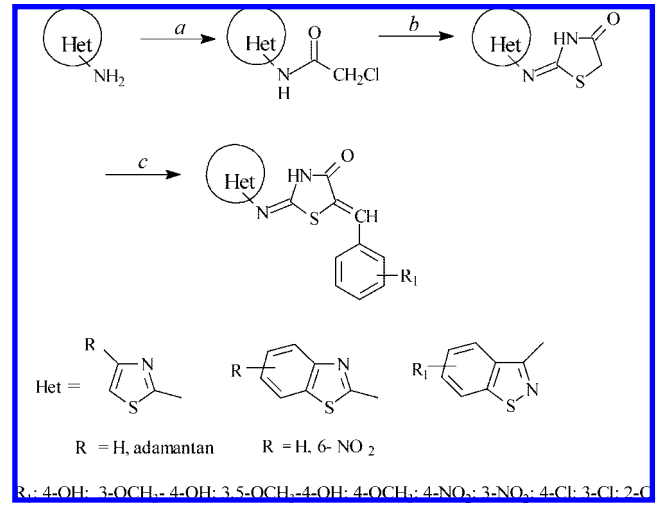
Appropriate thiazol-2-chloroacetamides or benzo[*d*]thiazol-2-yl/benzo[*d*]isothiazole chloracetamides were synthesized starting from 2-aminothiazoles/2-aminobenzothiazoles/aminobenzothiazoles/3-aminobenzo[*d*]isothiazoles as described in ref 29.

The first step of the synthetic procedure was carried out by reaction of appropriate amines with chloroacetyl chloride in DMF at room temperature for 2 h or by heating in dry benzene for 3 h.[29,30]

2-Chloro-*N*-(thiazole/benzo[*d*]thiazol-2-yl/benzo[*d*]isothiazol-3-yl)acetamides efficiently reacted with ammonium thiocyanate in refluxing ethanol to produce 2-(thiazol-2-ylimino)thiazolidin-4-ones or 2-(heteroarylimino)thiazolidin-4-ones.[28,31,32]

The final compounds were obtained by refluxing the previous products with commercially available aromatic aldehydes and anhydrous sodium acetate in glacial acetic acid (Scheme 1). The

Scheme 1. Synthesis of the Compounds[a]



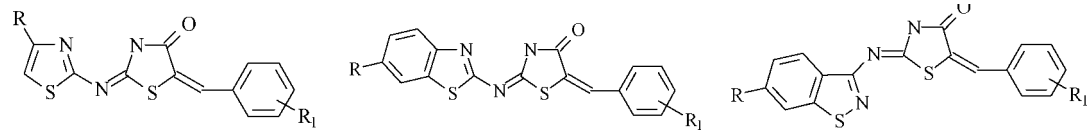
[a] Reagents and conditions: (a) $ClCOCH_2Cl$, *N*,*N*-DMF, room temp, 2 h; (b) NH_4SCN, EtOH, reflux, 1−3 h; (c) RC_6H_4CHO, MeCOOH, MeCOONa, reflux 2−4 h.

mechanism suggested for the formation of all synthesized compounds is described in our previous publications.[28,33]

All the new compounds were characterized by mp, elemental analyses, and spectroscopic data (^{1}H NMR, MS, and IR). The substitution position in the cyclocondensation step and the tautomeric structure of the 2-imino-5-arylidene-4-thiazolidinones were determined through the analysis of IR and ^{1}H NMR spectral data.[28,33] Benzo[*d*]thiazole and benzo[*d*]isothiazole derivatives show, in the ^{1}H NMR spectra, a NH proton at 12.00−12.87 ppm which is in accordance with a lactam proton and not with an imine proton (expected around 9.70 ppm). This observation accounts for the ring closure, as already discussed in our previous papers.[22] The feature of a γ-lactam heterocycle, in the solid state, is also supported by the IR spectral data (NH group band at 3100 cm^{-1} and a strong band at about 1690−1714 cm^{-1}) for the majority of the compounds. Compound **16** is an exception because it shows an absorption band around 3234 cm^{-1}, typical of a secondary amine.

Evaluation of SHP-2 Inhibition. All thiazolidin-4-one derivatives synthesized were tested for SHP-2 inhibitory action using human recombinant GST-fusion SHP-2 (Table 1). According to the results obtained, most of the tested compounds exhibited good inhibitory activity. It was found that the most active compound is 4-methoxy-substituted thiazole derivative **3**, as well as its benzothiazolyl-analogue **11** (K_i = 11.7 μM). In the series of thiazole derivatives, two subgroups are observed: compounds **3**, **2**, **8**, and **6**, with K_i values varying between 11.7 and 54.5 μM, and compounds **1**, **4**, **5**, and **7**, which are practically inactive, with K_i values varying between 388.0 and 1088.2 μM. As gathered by the results, the presence of a methoxy substituent strongly favors inhibitory action. Replacement of the methoxy group at the 4-position of the phenyl ring (**3**) with a hydroxy group (**1**) resulted in loss of activity (K_i = 388.0 μM). Moreover, addition of a methoxy group at the 3-position of the 4-hydroxy derivative led to compound **2** with increased inhibitory activity (K_i = 25.6 μM). The 2-Cl and 4-Cl derivatives also showed considerable inhibitory action (K_i values of 32.3 and 54.5 μM, respectively). In contrast, the 3-Cl derivative exhibited the lowest inhibition (K_i = 1088.2 μM), pointing out the importance of the position of the substituent in this case. The nitro derivatives of this series, **4** and **5**, are also practically inactive (K_i values of 473.6 and 547.5 μM,

Table 1. SHP-2 Inhibitory Action of Synthesized Compounds



compd	ring	R	R_1	K_i[a] (μM)
1	thiazole	H	4-OH	388.0
2	thiazole	H	3-OCH_3, 4-OH	25.6
3	thiazole	H	4-OCH_3	11.7
4	thiazole	H	4-NO_2	473.6
5	thiazole	H	3-NO_2	547.5
6	thiazole	H	4-Cl	54.5
7	thiazole	H	3-Cl	1088.2
8	thiazole	H	2-Cl	32.3
9	thiazole	4-phenyl	3-OCH_3, 4-OH	103.2
10	thiazole	4-adamantanyl	3-OCH_3, 4-OH	12.3
11	benzothiazole	H	4-OCH_3	11.7
12	benzothiazole	H	3,5-OCH_3, 4-OH	18.1
13	benzothiazole	6-NO_2	3,5-OCH_3, 4-OH	12.9
14	benzothiazole	H	4-NO_2	16.2
15	benzothiazole	H	3-NO_2	28.7
16	benzisothiazole	H	4-OCH_3	22.8
17	benzisothiazole	H	3-OCH_3, 4-OH	90.8

compd	IC_{50} (μM)
furanodictin A derivative **2a**	2.47[b]

[a] K_i values were the mean of two experiments. SD did not exceed 10% of the mean value. [b] IC_{50} value of furanodictin A was taken from the literature.[17]

respectively). Comparison of compound **10** ($K_i = 12.3$ μM) with compound **2** ($K_i = 25.6$ μM) showed that addition of the bulky, hydrophobic adamantanyl group at the 4 position of the thiazolyl ring (**10**) significantly increased inhibition. Interestingly, addition of a phenyl substituent at the same position (**9**, $K_i = 103.2$ μM) did not favor inhibitory action. Among compounds of benzo[*d*]thiazole series, 4-methoxy derivative **11** is the most active one, with activity equal to that of the thiazolyl analogue (**3**). Generally, the benzo[*d*]thiazolyl derivatives exhibited equal or better inhibitory action than thiazole derivatives, as shown by comparing compound **11** ($K_i = 11.7$ μM) to compound **3** ($K_i = 11.7$ μM), compound **14** ($K_i = 16.2$ μM) to compound **4** ($K_i = 474.0$ μM), and compound **15** ($K_i = 28.7$ μM) to compound **5** ($K_i = 548.0$ μM). Comparison of the 6-nitrobenzo[*d*]thiazolyl derivative **13** ($K_i = 12.9$ μM) with its benzo[*d*]thiazolyl analogue **12** ($K_i = 18.1$ μM) showed that the presence of the nitro group in the benzo[*d*]thiazolyl moiety further enhanced inhibitory activity. Among compounds of the benzo[*d*]isothiazolyl series, the 4-methoxy derivative **16** ($K_i = 22.8$ μM) caused stronger SHP-2 inactivation than the 3-methoxy-4-hydroxy derivative **17** ($K_i = 90.9$ μM). This is in accordance with its thiazolyl analogues, indicating the propitious addition of the methoxy group. In general, the benzo[*d*]isothiazoles tested were found to be less active compared to the thiazole analogues.

In order to analyze the mode of binding of different derivatives, docking studies for selected compounds were performed.

Docking. Docking analysis revealed that hydrogen bond (HB) formation and hydrophobic interactions are the most important factors affecting inhibitory action of compounds of the thiazole series. In general, compounds of the most active group (**3**, **2**, **8**, **6**) form more hydrogen bonds than compounds of the practically inactive group (**1**, **4**, **5**, **7**). Moreover, hydrophobic interactions are involved in the binding of the most active compounds.

All compounds of the active group of the thiazole series are oriented in the active center of the enzyme in a way that places the phenyl ring into the pocket consisting of the amino acids Lys366, Arg362, Glu361, Val360, Ser365, Tyr62, and Ser460.

Compound **3**, exhibiting the highest activity, forms four hydrogen bonds (Figure 3a). Hydrogen-bond interaction is observed between oxygen of the methoxy group at the 4-position of the phenyl ring and hydrogen of the NH group of the side chain of the amino acid Lys366 (distance 2.55 Å). Hydrogen of the NH group that connects the thiazole and thiazolidinone rings of the ligand forms a hydrogen bond with oxygen of the carbonyl group of amino acid Arg278 (distance 2.37 Å). Hydrogen of the OH group of the amino acid Tyr62 showed hydrogen-bond interaction with oxygen of the carbonyl group of the thiazolidinone ring (distance 2.84 Å), while the backbone NH group of the amino acid Lys280 formed a hydrogen bond with N of the thiazole ring (distance 2.58 Å). Moreover, hydrophobic interactions between the phenyl ring, the methyl group of the methoxy substituent, and the four-carbon chain of Lys 364 may occur.

The presence of the methoxy group at the 3-position and of an OH group at the 4-position of the phenyl ring in compound **2** causes a change in orientation of the compound in the active site of the enzyme (Figure 3b). Hydrogen of the NH group between the thiazole and thiazolidinone rings still forms a hydrogen bond with the oxygen of the carbonyl group of amino acid Arg278 (distance 1.83 Å), while the carbonyl group of the thiazolidinone ring fails to interact with the HO group of Tyr62. This carbonyl group now forms a hydrogen bond with oxygen of the methoxy group at the 3-position of the phenyl ring (distance 2.49 Å). A third hydrogen bond is formed between hydrogen of the OH group at the 4-position of the phenyl ring and oxygen of the carbonyl group of the backbone peptide bond of amino acid Glu361 (distance 2.49 Å). Hydrophobic interactions between the methyl group of methoxy substituent and the four-carbon chain of Lys 364 may be involved in complex stabilization. The lower number of hydrogen bonds in the case of compound **2** explains its higher K_i value compared to the most active derivative **3**.

The three hydrogen bonds observed in the 4-Cl derivative, compound **6** (Figure 3c), are formed between the NH group that connects the thiazole and thiazolidinone rings and the oxygen of the carbonyl group of Arg278 (distance 1.27 Å), the oxygen of the carbonyl group of the thiazolidinone ring, and the OH group of Tyr62 (distance 3.11 Å) and between the N

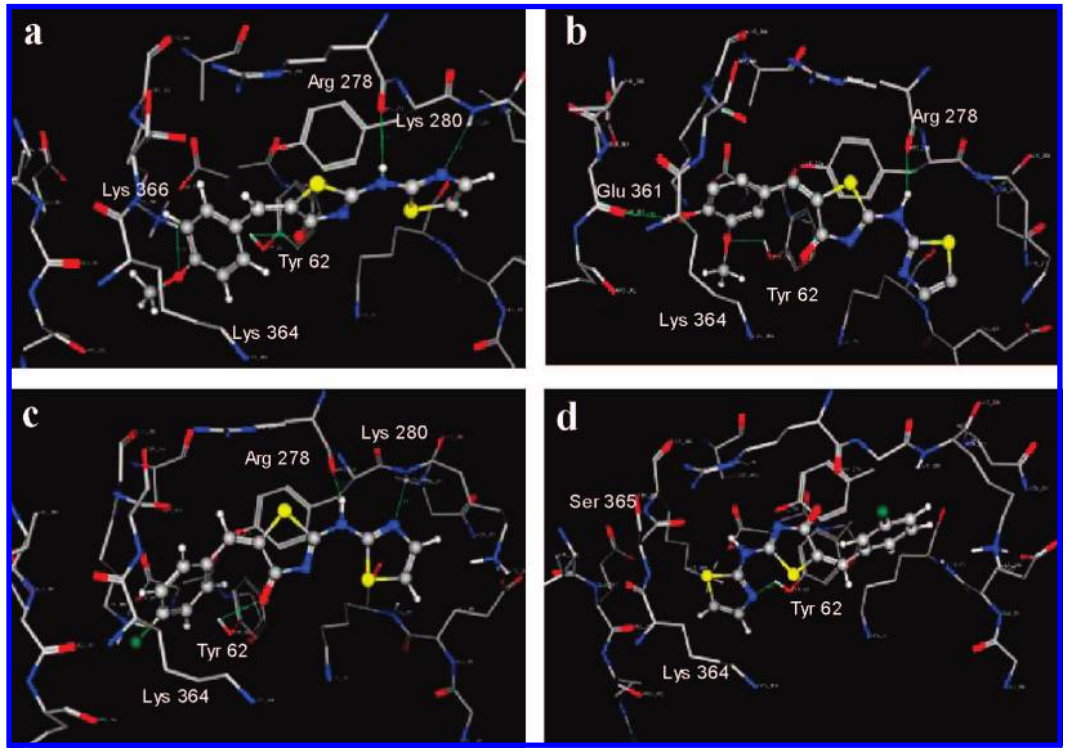


Figure 3. (a) Docking of compound **3**, (b) docking of compound **2**, (c) docking of compound **6**, and (d) docking of compound **7** in the active site of human SHP-2. Green lines represent H-bonding.

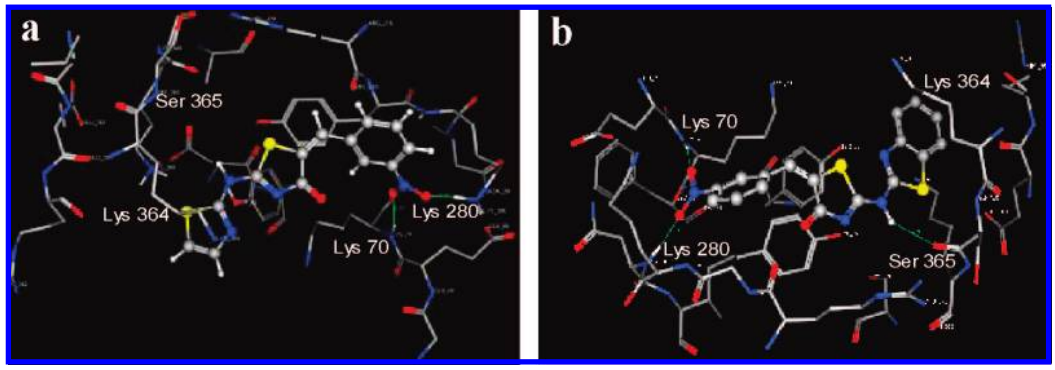


Figure 4. (a) Docking of compound **5** and (b) docking of compound **15** in the active site of human SHP-2. Green lines represent H-bonding.

atom of the thiazole ring and the backbone NH group of Lys280 (distance 2.42 Å). Orientation of the phenyl ring does not favor any hydrophobic interaction. The number of hydrogen bonds and the lack of hydrophobic interactions are probably responsible for the lower activity of this compound compared to the most active compounds of this series (**3** and **2**).

The presence of the Cl substituent at the 3-position of the phenyl ring, compound **7**, forces it to be placed in the opposite orientation in the active site compared to compounds **6** and **8**, where Cl substituent is in positions 4 and 2, respectively. Docking results showed that the phenyl ring with 3-Cl substituent is placed in the cavity comprising amino acids Glu69, Lys70, Lys280, and Asn281, while the thiazole and thiazolidinone rings are in the vicinity of the amino acids Tyr62, Arg278, Tyr279, Lys364, Lys366, and Ser460 (Figure 3d). The only hydrogen bond observed in this orientation is formed between hydrogen of the OH group of Tyr62 and N of the thiazole ring (distance 2.71 Å). Moreover, no hydrophobic interactions are favored. These results explain the huge decrease in activity of the 3-Cl derivative (**7**) compared to 4-/2-Cl derivatives.

The same upside-down orientation is observed in all compounds of the inactive group of the thiazole series bearing a nitro substituent at the 4 or 3 position of the phenyl ring (compounds **4** and **5**, respectively) or a OH group at the 4-position (compound **1**). A low number of hydrogen bonds and lack of hydrophobic interactions are observed in all cases. It is interesting to mention that this orientation is also observed in the benzo[*d*]thiazole derivatives that exhibit significant activities compared to their thiazole analogues. The number of hydrogen bonds and the hydrophobic interactions of benzo[*d*]thiazole moiety are probably responsible for better activity in this case.

Docking of the 3-nitrothiazole derivative, compound **5**, showed that one oxygen atom of the NO_2 group forms a hydrogen bond with the NH group of the side chain of Lys 280 (distance 1.64 Å), while the other oxygen atom interacts with the hydrogen of the backbone NH of Lys70 (distance 2.38Å) (Figure 4a). Docking of the benzo[*d*]thiazolyl analogue **15** revealed that oxygen atoms of the NO_2 group also form hydrogen bonds with the side chain NH of Lys 280 and the

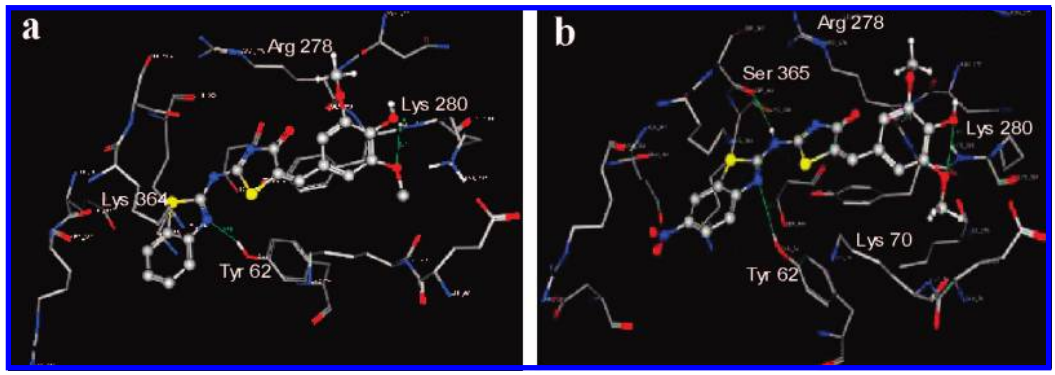


Figure 5. (a) Docking of compound **12** and (b) docking of compound **13** in the active site of human SHP-2. Green lines represent H-bonding.

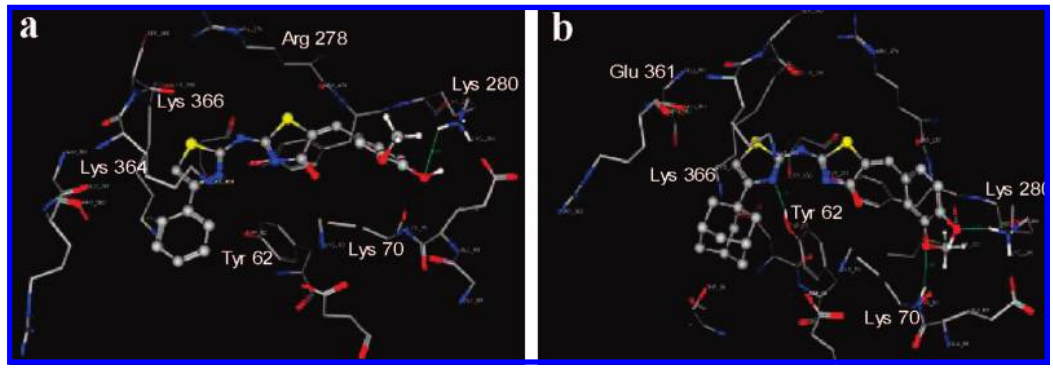


Figure 6. (a) Docking of compound **9** and (b) docking of compound **10** in the active site of human SHP-2. Green lines represent H-bonding.

backbone NH of Lys 70 (distances 1.94 and 1.62 Å, respectively). However, a minor rearrangement due to the presence of the benzo[*d*]thiazole results in an extra hydrogen bond formation between the NH group that connects the thiazole and thiazolidinone rings and the backbone carbonyl group of Ser365 (distance 2.70) (Figure 4b). Docking studies of the thiazole and benzo[*d*]thiazole 4-nitro derivatives (compounds **4** and **14**, respectively) revealed that both oxygen atoms of the NO_2 group form hydrogen bonds with the NH group of the side chain of Lys280 (distances of 1.99 and 2.44 Å in compound **4** and of 1.58 and 2.54 Å in compound **14**). Hydrophobic interactions between the benzo[*d*]thiazole ring system and the four-carbon chain of Lys364 favor complex stabilization in this case.

The positive influence of the nitro group at the 6-nitrobenzo[*d*]thiazole derivative **13** is well explained by docking analysis of compounds **12** and **13** (Figure 5). The presence of the nitro group forces the compound to an orientation that allows formation of an extra hydrogen bond compared to the benzo[*d*]thiazolyl analogue **12**. Docking of compound **12** showed that the 3,5-OCH_3-4-OH substituted phenyl ring is oriented in the pocket consisting of Glu69, Lys70, Asn277, Arg278, Tyr279, and Lys280, while the benzo[*d*]thiazole and thiazolidinone moieties are in the vicinity of Tyr62, Glu361, Arg362, Tyr279, Lys364, Ser365, Lys366, and Ser460. Hydrogen bond interactions are observed between the 4-OH and 5-OCH_3 groups of the phenyl ring and the backbone NH of Lys280 (distances of 1.81 and 2.34 Å, respectively) and between the OH group of Tyr62 and nitrogen of the benzo[*d*]thiazole moiety (distance 2.51 Å) (Figure 5a). Docking of compound **13** revealed that the 3,5-OCH_3-4-OH substituted phenyl ring is placed in the pocket consisting of Tyr62, Glu69, Lys70, Arg278, Tyr279, and Lys280 while the 6-NO_2 benzo[*d*]thiazole group is in proximity to amino acids Tyr62, Glu361, Arg362, Lys364, Ser365, Lys366, and Ser460. The 4-OH and 5-OCH_3 groups show HB interactions with a backbone NH of Lys280 (distances of 2.19 and 2.29 Å, respectively), while the OH group of the amino acid Tyr62 interacts with the nitrogen of the thiazole ring of the 4-nitrobenzo[*d*]thiazole moiety (distance 2.72 Å). An extra hydrogen bond is formed between hydrogen of the NH connecting the benzo[*d*]thiazole and thiazolidinone moieties and oxygen of the carbonyl group of the amino acid Ser365 (distance 2.34 Å) (Figure 5b).

Docking of the 4-phenyl/adamantanyl-thiazole derivatives **9** and **10** revealed a general orientation similar to the compounds of the benzo[*d*]thiazole series. However, a minor difference in orientation due to the presence of the bulky adamantanyl group results in formation of two more hydrogen bonds compared to the 4-phenylthiazolyl analogue where only one hydrogen bond is formed (Figure 6). This explains well the difference in inhibitory activities of these compounds. More precisely, docking of 4-phenylthiazole derivative **9** showed that the 3-OCH_3-4-OH substituted phenyl ring is placed in the pocket consisting of Gly68, Glu69, Lys70, and Lys280, whereas the thiazole and thiazolidinone rings are in proximity to the amino acids Tyr62, Tyr279, Lys364, Ser365, Lys366, and Ser460. Hydrogen bond interaction is observed between oxygen of the 4-OH substituent of the phenyl ring of the compound and nitrogen of the side chain NH group of Lys280 (distance 2.65 Å). Docking of 4-adamantanylthiazole derivative **10** showed that the substituted phenyl ring is located in the pocket consisting of Glu69, Lys70, Arg278, Tyr279, and Lys280, while thiazole and thiazolidinone rings and the 4-adamantanyl group are placed in the cavity of Gln57, Thr59, Asp61, Tyr62, Asp64, Lys70, Tyr279, Glu361, Arg362, Lys364, Ser365, and Lys366. The 4-OH group of the phenyl ring forms a hydrogen bond with the NH group of the side chain of Lys280 (distance 1.74Å). Moreover, hydrogen bonds are observed between oxygen of the 3-OCH_3 group of the phenyl ring and hydrogen of the backbone NH of Lys70 (distance 2.19 Å) and between OH group of Tyr62 and nitrogen of the thiazole ring (distance 2.16 Å).

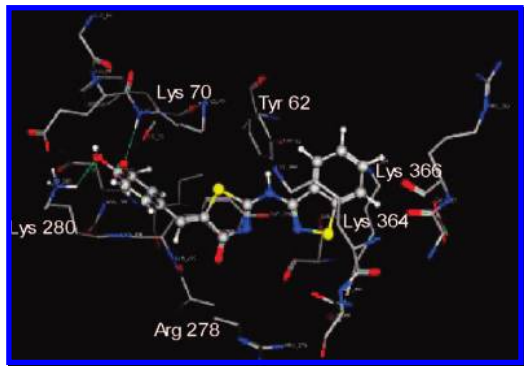


Figure 7. Docking of compound **17** in the active site of human SHP-2. Green lines represent H-bonding.

Upside-down orientation is observed in benzo[*d*]isothiazole derivatives, as well, resulting in a low number of hydrogen bonds and reduced activity of the compounds. Docking studies of compound **17** (Figure 7), the benzo[*d*]isothiazole analogue of the thiazole derivative **2**, showed that the phenyl ring with the 3-OCH_3 and 4-OH substituents is placed in the cavity comprising Glu69, Lys70, Phe71, Arg278, Tyr279, Lys280, and Asn281, while the thiazole and thiazolidinone rings are in proximity to amino acids Tyr62, Arg278, Tyr279, Glu361, Arg362, Lys364, Ser365, Lys366, and Ser460. Hydrogen bond interaction has been observed between the oxygen of the methoxy group of the phenyl ring and the backbone NH of Lys70 (distance 2.11 Å), while the oxygen of the OH group of the ligand interacts with the hydrogen of the NH of side chain of Lys280 (distance 1.97Å). This orientation does not strongly favor hydrophobic interactions. The low number of hydrogen bonds and lack of strong hydrophobic interaction explains the lower activity of compound **17** compared to its thiazole analogue (**2**).

In all cases, stable complex formation was favored by the placement of a hydrophobic group in the vicinity of amino acids Tyr62, Glu361, Arg362, Lys364, Ser365, Lys366, and Ser460. Hydrogen bond interactions between the compounds and aminoacids of the enzyme further justify the complex stabilization explaining differences in activity. Lys 280, Tyr62, and Arg 278 are the amino acids mainly involved in this process. Lys280 is implicated in hydrogen bond formation of most of the active derivatives (8 out of 11). It interacts with the substituents of the phenyl ring of benzo[*d*]thiazoles with the exception of benzo[*d*]thiazolyl derivative **11**, where it forms a hydrogen bond with the carbonyl group of the thiazolidinone ring. In compounds of the thiazole series, HB formation between Lys280 and the thiazolyl ring was observed. Tyr62 is also involved in hydrogen bond interactions with the thiazole or thiazolidinone ring of many active compounds (7 out of 11), while the NH group connecting the thiazole and thiazolidinone rings interacts with Arg278. Lys 280, involved in stabilization of most complexes, was one of the tree amino acids referred to interact with inhibitor NSC-87877, according to the literature.[18]

Conclusions

As a result of our research, a number of new SHP-2 inhibitors have been found and a new class of inhibitors have emerged. The tested compounds are the first thiazole/thiazolidinone derivatives found to have SHP-2 inhibitory action. Eleven out of seventeen compounds have K_i values lower than 54.5 μM, whereas four of them have K_i values lower than 12.9 μM. 5-(4-Methoxybenzylidene)-2-(thiazol-2-ylimino)thiazolidin-4-one and 5-(4-methoxy-benzylidene)-2-(benzo[*d*]thiazol-2-ylimino)thiazolidin-4-one exhibited the best inhibitory action with $K_i = 11.7$ μM. In general, benzo[*d*]thiazole derivatives showed equal/better inhibitory activity than their thiazole analogues. Docking results explain inhibitory action well. In all cases, stable complex formation was favored by the placement of a hydrophobic group in the vicinity of amino acids Tyr62, Glu361, Arg362, Lys364, Ser365, Lys366, and Ser460. Hydrogen bond formation and hydrophobic interactions are involved in stabilization.

Unfortunately, a direct comparison of the activity of the best compounds of these series with NSC-87877, the most potent SHP-2 inhibitor known till now ($IC_{50} = 0.318$ μM) is not possible because of the use of different methods of SHP-2 activity evaluation (different substrate and substrate concentration).

Toxicity of some of our compounds has been tested during a previous investigation, and results were very encouraging.[34] Since investigation on SHP-2 inhibitors targets the development of potent drugs for the chronic treatment of individuals bearing PTPN11 mutations, low or no toxicity of the compounds is essential. Furthermore, anti-inflammatory activity of some of the compounds was observed.[34] Because Noonan syndrome patients often suffer from chronic inflammations, which may be the reason for the deafness observed in some individuals, compounds of this series may possess an extra beneficial property. Of course, more experiments have to be done for the determination of selectivity of the compounds. Moreover, new derivatives with improved properties could be designed taking into account all previous results.

Experimental Section

Materials. Synthetic starting material, reagents, and solvents were purchased from Aldrich or Fluka. All the solvents were reagent grade and dried prior to use. Melting points were determined with a Boetius apparatus and are uncorrected. Elemental analysis was performed in the analytical laboratory of Dipartimento Farmaceutico, Università di Parma, on a ThermoQuest (Italia) FlashEA 1112 elemental analyzer, for C, H, N, and S. The found values for C, H, N, S were always ±0.4% of the theoretical ones. IR spectra were taken as KBr pellets on a Jasco FT-IR 300E spectrophotometer (Jasco Ltd., Tokyo, Japan) or on Perkin-Elmer spectrophotometer, and the reported wavenumbers are given in cm^{-1}. ^1H NMR spectra, in DMSO-d_6 solutions, were recorded on a Bruker AC 300 instrument at 298 K. Chemical shifts are reported as δ (ppm) relative to TMS as internal standard. Mass spectra were recorded on a VG-250 spectrometer (VG Laboratories., Tritech England) at 70 eV. The progress of the reactions was monitored by thin layer chromatography using F_{254} silica gel precoated sheets (Merck, Darmstadt, Germany). Human recombinant GST-fusion SHP-2 was purchased by Calbiochem.

Chemistry. Synthesis of 4-adamantyl-2-aminothiazole. To a solution of 1-adamantyl bromomethyl ketone, **I** (257 mg, 1 mmol), in 5 mL of isopropanol, a suspension of thiourea (152 mg, 2 mmol) in 10 mL of isopropanol was added. The mixture was stirred for half an hour. After this time the resulting solution was poured into a solution of sodium carbonate, and the precipitate formed was filtered and dried to give, after recrystallization from ethyl acetate, 220 mg (94%) of pure product, **II**. Mp 215−215.5 °C. IR(KBr): ν = 3050 cm^{-1} (N−H), 1650 cm^{-1} (C═O).

Chloracetylchloride of 4-adamantyl-2-aminothiazole, chloracetamidothiazoles/benzothiazoles/benzoisothiazoles were prepared as previously described.[30]

General Procedure for Synthesis of 4-Adamantyl-2-thiazolylimino-5-arylidene-4-thiazolidinones. A well-stirred solution of 0.8 g of 2-(thiazol-2-ylimino)thiazolidin-4-one (4 mmol) in 35 mL of acetic acid was buffered with sodium acetate (8 mmol) and added to the appropriate arylaldehyde (6 mmol). The solution was refluxed for

4 h and then poured into ice-cold water. The precipitate was filtered and washed with water, and the resulting crude product was purified by recrystallization from dioxane.

5-(4-Hydroxy-3-methoxybenzylidene)-2-(4-phenylthiazol-2-ylimino)thiazolidin-4-one (9). Reaction time: 4 h. Yield: 58.9%; mp 227−29 °C (dioxane). TLC: eluent = toluene/dioxane/acetic acid 90/10/5. IR (KBr): ν = 3111 (N−H), 1735 (C═O), 1581 (N═C) cm^{-1}. MS: *m/e* 409.06 (100%), 410.06 (21.9%), 411.06 (9%).

5-(4-Hydroxy-3-methoxybenzyliden)-2-(4-adamantan-1-yl-thiazol-2-ylmino)thiazolidin-4-one (10). Reaction time: 4 h. Yield: 83.6%; mp 288−290 °C (dioxane). TLC: eluent = toluene/dioxane/acetic acid 90/10/5. IR (KBr): ν = 3120 (N−H), 1581 (C═O), 1597 (N═C) cm^{-1}. ^{1}H NMR (DMSO-d_6 δ, ppm): 1.62−1.97 (m, 15H adam), 3.76 (s, 3H, OCH_3), 6.861−6.870 (m, 2H, ArH and thiaz), 7.147−1.152 (m, 2H, C_2−C_6 ArH), 7.551 (s, 1H, ═CH), 9.938 (s, 1H, OH), 12.461 (s 1H, NH). MS: *m/e* 467.13 (100%), 468.14 (26.4%), 469,13 (9.4%).

5-(4-Hydroxy-3,5-dimethoxybenzylidene)-2-(benzo[*d*]thiazole-2-ylimino)thiazolidin-4-one (12). Reaction time: 4 h. Yield: 81.5%; mp 278−280 °C (dioxane). TLC: eluent = toluene/dioxane/acetic acid 90/10/5. IR (KBr): ν = 3120 (N−H), 1700 (C═O), 1592 (N═C) cm^{-1}. MS: *m/e* 413.05 (100%), 414.05 (23%), 415,05 (9.4%).

5-(4-Hydroxy-3,5-dimethoxybenzylidene)-2-(6-nitrobenzo[d]thiazole-2-ylimino)thiazolidin-4-one (13). Reaction time: 4 h. Yield: 56.9%; mp 271−273 °C (dioxane). TLC: eluent = toluene/dioxane/acetic acid 90/10/5. IR (KBr): ν = 3457 (OH), 3089 (N−H), 1720 (C═O), 1610 (N═C, 1345 (NO_2) cm^{-1}. MS: *m/e* 458.04 (100%), 459.04 (20.7%), 460,03 (9%).

Biological Evaluation.[18,35] SHP-2 inhibitory activity was tested using human recombinant GST-fusion SHP-2 (Calbiochem). Incubation was carried out at 25 °C for 45 min in a 100 μL of reaction mixture containing 20 mM Tris-HCl, pH 7.0, 50 mM NaCl, 1 mM DTT, 1 mM EDTA, 0.5 mg/mL BSA, and 0.13 U of the enzyme. An amount of 10 μL of each compound dissolved in DMSO was added and was preincubated with the enzyme mixture for 15 min at room temperature before addition of the substrate. The substrate, *p*-nitrophenyl phosphate, was used at concentrations of 2.5, 5, 10, 20, and 40 mM. Enzyme activity was estimated by measuring the absorbance at 405 nm with appropriate corrections for absorbance of the compounds.

Docking. In order to analyze the mode of binding of 2-thiazolylimino/heteroarylimino-5-arylidene-4-thiazolidinones, all synthesized compounds were docked to SHP-2 protein tyrosine phosphatase using the GOLD[36] 3.0.1 software running on a Windows based PC. The three-dimensional coordinates of SHP-2 were taken from the Protein Data Bank (PDB code 2Shp). Since the only available report was the one concerning docking studies of NSC-87877 inhibitor to SHP-2[18] using the GLIDE program (grid-based ligand docking from energetics),[37] NSC-87877 has been used as a standard in our docking studies.

Preparation of Protein and Ligand for Docking Studies. Since the X-ray crystallographic structure of the protein is available without the ligand, NSC-87877 inhibitor was docked[18] to the SHP-2 protein and this protein−ligand complex was minimized using the force field MMFF94s available in the software MOE up to a gradient of 0.01 kcal/(mol Å). The backbone atoms of the protein SHP-2 were fixed, and the hydrogens were added during the minimization process. This minimized protein−ligand complex was used for the docking studies of the 17 compounds (Table 1). In order to further confirm that this protein structure can be used for the docking of the compounds, the ligand NSC-87877 was docked using the GOLD 3.0.1 software and it was observed that the ligand−protein complex so generated was quite similar to the ligand−protein complex reported in the literature.[18]

Supporting Information Available: Elemental analysis results and IR spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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