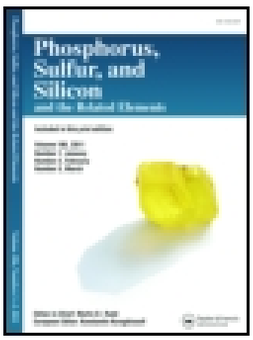

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Synthesis and biological activity evaluation of new thiazolidinone-diclofenac hybrid molecules

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ABSTRACT

A novel series of [2-(2,6-dichlorophenylamino)-phenyl]-acetic acid *N*-3-(substituted)-4-thiazolidin-5-ylidenemethyl-hydrazide derivatives has been designed and synthesized. The structures of synthesized compounds were confirmed by their ^{1}H NMR, ^{13}C NMR and LCMS spectroscopic data. Target compounds were screened for their *in vitro* anticancer activity according to US NCI protocols, *in vitro* trypanocidal activity toward *Trypanosoma brucei brucei* (*Tbb*) and evaluated for anti-inflammatory activity on the carrageenan edema model in rats. Biological screening data led to identification of compounds **3.3** ([2-(2,6-dichloro-phenylamino)-phenyl]-acetic acid *N*-(4-oxo-2-thioxo-thiazolidin-5-ylidenemethyl)-hydrazide) and **3.7** ([2-(2,6-dichloro-phenylamino)-phenyl]-acetic acid *N*-(4-oxo-2-thioxo-3-(3-trifluoromethylphenyl)thiazolidin-5-ylidenemethyl)-hydrazide) which demonstrated moderate antitumor activity on the non-small-cell lung cancer NCI-H522 and colon cancer HCT-116 cell lines. Several hit compounds (**3.2**, **3.4**) exhibited the promising and significant inhibition growth of the parasites at micromolar concentrations (IC_{50} values of 4.8 and 7.06 μM, respectively). The synthesized compounds also demonstrated considerable anti-inflammatory effect comparable to the reference non-steroidal anti-inflammatory drugs (NSAIDs) diclofenac sodium or ketorolac tromethamine.

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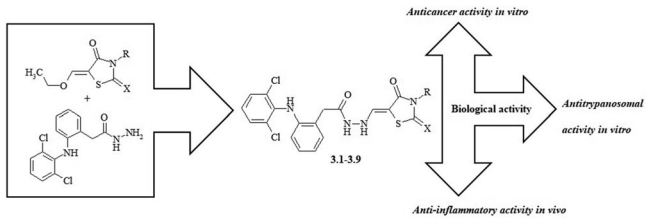
Diclofenac; 4-thiazolidinones; anticancer; antitrypanosomal; anti-inflammatory

GRAPHICAL ABSTRACT



Introduction

Functionalized nitrogen- and sulfur-containing heterocycles, especially thiazoles/thiazolidinones, have been historically developed in medicinal and pharmaceutical chemistry due to their various biological activities and clinical applications.[1–3] Thus, thiazole-based derivatives are a well-known class of biologically active substances that became the basis for the creation of various medicinal agents, such as penicillin and monobactam antibiotics, sulfa drug sulfathiazole, antifungal and antiparasitic agent thiabendazole, hypoglycemic thiazolidinones (Pioglitazone and its analogs), H_2-receptor antagonist (Nizatidine), aldose reductase inhibitors (Epalrestat), dual inhibitors of COX-2/5-LOX (Darbufelone) and loop diuretic (Etozoline). In addition, the structural features of the 4-thiazolidinone core allowed access a wide range of functionally substituted and polycyclic derivatives with polypharmacological profiles.[4–9] Thus, among thiazolidinones a set of potent antimicrobial,[10,11] antiviral,[12,13] antioxidant,[14] anticancer[1,15] and anti-inflammatory agents[16,17] have been identified. In addition, thiazolidinone structural-type compounds are considered as cyclic analogs of well-known antitripanosomal thiourea/thiosemicarbazones and have shown high affinity to the trypanosome-validated targets.[18,19] Due to the importance of these compounds, data have been also reported for 4-thiazolidinones as

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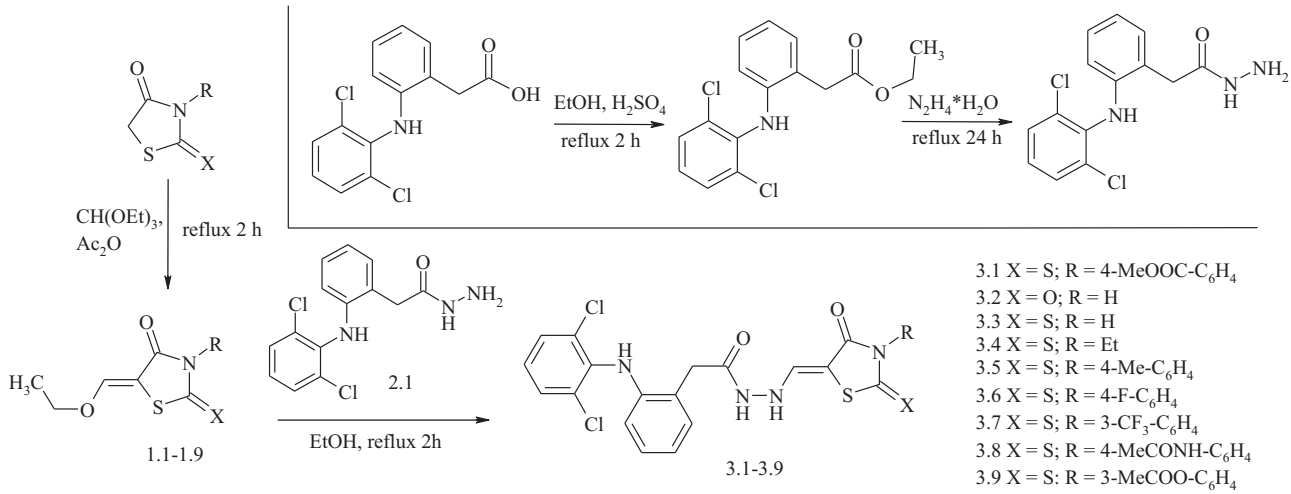

Scheme 1. Synthesis of [2-(2,6-dichloro-phenylamino)-phenyl]-acetic acid *N*-3-(substituted)-4-thiazolidin-5-ylidenemethyl-hydrazide derivatives **3.1–3.9**.

modulators of NF-kB signaling pathway,[20] which has a major role in the pathogenesis of inflammatory diseases and cancer.[21] The literature contains several reports about pharmacophore hybrid approach for the synthesis of multi-targeted agents.[22,23] It is well known that diclofenac sodium is an effective non-steroidal anti-inflammatory drug (NSAID), therapeutically used in inflammatory and painful diseases, and it possesses a wide spectrum of another activities, especially antimicrobial,[24] anticancer[25] and antioxidant[26] properties. As a result, the purpose of our work was the design and synthesis of hybrid molecules by linking the main structural unit of the 4-thiazolidinone ring system with the fragments of diclofenac using hybrid pharmacophore approach and examined their anti-inflammatory, antitumor and trypanocidal activities *in vitro*.

Results and discussion

Chemistry

The general method for the synthesis of the target derivatives is depicted in Scheme 1. Firstly, a set of 5-ethoxy-4-thiazolidinones was synthesized via a known approach based on the reaction of 4-thiazolidinones and triethyl orthoformate.[15] The reactions were performed in acetic anhydride medium. The starting 2-[2-(2,6-dichloroanilino)phenyl]aceto hydrazide was obtained according to the literature.[27] 5-Ethoxy-4-thiazolidinones was converted into appropriate enhydrazines **3.1–3.9** in the reaction with 2-[2-(2,6-dichloroanilino)phenyl]aceto hydrazide in an ethanol medium. The structures of the synthesized compounds were confirmed by elemental analysis and spectroscopic data (^{1}H NMR, ^{13}C NMR and LCMS). In ^{1}H NMR spectra, the characteristic secondary amine proton of diclofenac fragment appears as a singlet at δ ~7.48–9.87 ppm and methylene protons (Ar-CH_2-C-) assigned a singlet at δ ~3.65–3.88 ppm, respectively. The signals of NH protons of the methylenehydrazo group resonated as two broad singlets between 9.87 and 11.65 ppm. The CH protons of the methylene group (=CH) appear as a singlet at δ 7.50–7.84 ppm. In ^{13}C NMR spectra, the characteristic signals of (thio)carbonyl carbons at δ ~151.0–178.7 ppm and the signals of methylene group of diclofenac fragment (36.2–39.1 ppm) are observed. The signals between δ 113.5 and 162.8 ppm were assigned to the aromatic carbons, while the peaks of methylene group appeared between δ 142.3 and 147.0 ppm, respectively.

In vitro evaluation of the anticancer activity

Taking into account the results of previous investigations of thiazolidinones and structure-related analogs,[15,28] a series of 5-enhydrazine-4-thiazolidinones were evaluated for their *in vitro* anticancer activity. Thus, compounds **3.3**, **3.6**, **3.7** and **3.8** were selected by the National Cancer Institute (NCI) Developmental Therapeutic Program (www.dtp.nci.nih.gov) and evaluated for the antitumor activity at 10^{-5}M concentration toward a panel of approximately 60 cancer cell lines. The human tumor cell lines were representing leukemia, melanoma, lung, colon, central nervous system, ovarian, renal, prostate and breast cancers. Anticancer assays were performed according to the NCI protocol, which is described elsewhere.[29–31] The compounds were added at a single concentration, and the cell cultures were incubated for 48 h. The results for each compound are reported as the percentage of growth (GP%) of treated cells when compared with untreated control cells. The screening results are shown in Table S1 (supplemental materials). The tested compounds did not show significant activity in almost all cancer cell lines. Nevertheless, slight *in vitro* cytostatic effect was observed on the leukemia CCRF-CEM (GP% = 56.82%) for compound **3.3** and on the non-small-cell lung cancer NCI-H522 (GP% = 54.32%) and colon cancer HCT-116 (GP% = 57.71%) cell lines for compound **3.7**.

Antitrypanosomal activity

The antitrypanosomal activity of the novel thiazolidinone-diclofenac hybrid molecules **3.1–3.9** was studied in *in vitro* assay toward *Trypanosome brucei brucei* (Tbb). The IC_{50} values were calculated based on at least three independent experiments. In general, the synthesized compounds

inhibited the parasites growth at micromolar concentrations (supplemental materials Table S2). Thus, among the tested derivatives the most active compounds were found to be **3.2** and **3.4** with IC_{50} values of 4.8 and 7.06 μM, respectively. The SAR study revealed that the level of antitrypanosomal activity of synthesized compounds does not depend on substituents at C3 and C5 of thiazolidinone core. It should be noted that the hydrazine moiety in the C5 position of the basic heterocycle exhibited significantly lower impact on activity and selectivity against *Trypanosoma* species compared to previously described 4-thiazolidinone-2-hydrazones.[18]

Anti-inflammatory activity evaluation in vivo

The *in vivo* anti-inflammatory activity of the synthesized compounds was assessed by using the functional model of carrageenan-induced rat paw edema.[32,33] All tested compounds exhibited different protection against the carrageenan-induced paw edema (supplemental materials Table S3). Evaluation of anti-inflammatory activity indicated that compound **3.5** showed no significant decrease in edema with inhibition rate at the level 29.2% as compared to control group. The compounds **3.1**, **3.6** and **3.8** possessed the anti-inflammatory activity in the range of 39.5%–40.8% which is comparable with the effect of reference compound diclofenac sodium or ketorolac tromethamine. The SAR study revealed that the level of anti-inflammatory activity of synthesized compounds depends on substituents at 3-position of 2-thioxo-1,3-thiazolidin-4-one core. The presence of methyl benzoate or *N*-methylbenzamide substituents in the C-3 position of basic heterocycle improved the anti-inflammatory activity in comparison with another thiazolidinone structural-type compounds. Thus, for 5-enhydrazine-4-thiazolidinones the anti-inflammatory activity increases in the row of the following substituents: 4-Me-C_6H_4 < 4-MeOOC-C_6H_4 < 4-MeCONH-C_6H_4 < 4-F-C_6H_4.

Conclusion

We herein report the synthesis, spectral studies and pharmacological evaluation of a novel series of [2-(2,6-dichloro-phenylamino)-phenyl]-acetic acid *N*-3-(substituted)-4-thiazolidin-5-ylidenemethyl-hydrazide derivatives (**3.1**–**3.9**). Two tested compounds display moderate antitumor activity against the non-small-cell lung and colon cancer cell lines. The trypanocidal activity study of the synthesized compounds allowed to identify the most active derivatives [2-(2,6-dichloro-phenylamino)-phenyl]-acetic acid *N*-(2,4-dioxo-thiazolidin-5-ylidenemethyl)-hydrazide **3.2** and [2-(2,6-dichloro-phenylamino)-phenyl]-acetic acid N-(3-ethyl-4-oxo-2-thioxo-thiazolidin-5-ylidenemethyl)-hydrazide **3.4** with the IC_{50} values of 4.8 and 7.06 μM, respectively. The anti-inflammatory activity assay identified the active compounds **3.1**, **3.6** and **3.8** with 39.5%–40.8% protection against carrageenan-induced rat paw edema. The analysis of structure–activity relationships revealed that the level of biological activity (anticancer, antitrypanosomal and anti-inflammatory) of synthesized thiazolidinone-diclofenac hybrid molecules depends on substituents at 3-position of 2-thioxo-1,3-thiazolidin-4-one core. The biological tests of such 5-enhydrazine-4-thiazolidinones with diclofenac fragment in the molecules revealed the necessity for further investigation of anticancer, antitrypanosomal and anti-inflammatory activities for the construction of novel drug candidates with better pharmacological profiles.

Experimental section

Chemistry

General

All materials were purchased from commercial sources and used without purification. Melting points were measured in open capillary tubes and are uncorrected. Elemental analyses were performed using Perkin-Elmer 2400 CHN analyzer (Waltham, MA, USA). The ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on Varian Mercury 400/100 MHz instrument (Varian Medical Systems, Palo Alto, CA, USA) in DMSO-d_6 using tetramethylsilane as an internal standard. The purity of all obtained compounds was checked by thin-layer chromatography (eluent benzene:EtOAc 1:1). The supplemental materials contain sample ^{1}H, ^{13}C NMR and LS-MS spectra for products 3 (Figures S1–S21).

General procedure for the synthesis of [2-(2,6-dichloro-phenylamino)-phenyl]-acetic acid N-3-(substituted)-4-thiazolidin-5-ylidenemethyl-hydrazides (3.1–3.9)

A mixture of 2-[2-(2,6-dichloroanilino)phenyl]aceto hydrzide **2.1** (10 mmol) with the appropriate 5-ethoxy-4-thiazolidinones **1.1**-**1.9** (10 mmol) was refluxed for 2 h in the ethanol (10 mL). The obtained solid products were filtered off, washed with ethanol and recrystallized from the appropriate solvent.

Acetic acid 4-[5-(N-{2-[2-(2,6-dichloro-phenylamino)-phenyl]-acethyl}-hydrazinomethylene)-4-oxo-2-thioxo-thiazolidin-3-yl]-phenyl ester (***3.1***). Yield 83%, yellow powder, mp 163–164 °C (AcOH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.24 (s, 3H, CH_3), 3.86 (s, 2H, CH_2), 6.31 (d, 1H, J = 7.7 Hz, arom.), 6.91 (t, 1H, J = 7.7 Hz, arom.), 7.09 (t, 1H, J = 7.7 Hz, arom.), 7.19 (t, 1H, J = 7.9 Hz, arom.), 7.32–7.36 (m, 2H, arom.), 7.41 (m, 2H, arom.), 7.46–7.48 (m, 3H, NH, arom.), 7.53 (d, 1H, J = 8.1 Hz, arom.), 7.66 (s, 1H, CH), 10.63 (s, 1H, NH), 11.65 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 21.3 (CH_3), 37.5 (CH_2), 116.8, 121.4, 123.0, 124.4, 125.8, 128.2, 129.7, 130.0, 130.4, 131.2, 133.7, 137.5, 141.9, 143.3 (=CH), 151.0 (C = O), 169.5 (C = O), 171.9 (C = O), 172.0 (C = S). Anal. Calcd for $C_{26}H_{20}Cl_2N_4O_4S_2$: C, 53.16; H, 3.43; N, 9.54. Found: C, 53.08; H, 3.49; N, 9.62. ESI-MS m/z 586/588 $(M + H)^+$.

[2-(2,6-Dichloro-phenylamino)-phenyl]-acetic acid N-(2,4-dioxo-thiazolidin-5-ylidenemethyl)-hydrazide (***3.2***). Yield 68%, gray powder, mp 293–294 °C (DMF:AcOH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.65 (s, 2H, CH_2), 6.29 (d, 2H, J = 8.0 Hz, arom.), 6.86 (t, 1H, J = 7.2 Hz, arom.), 7.05 (t, 1H, J = 7.2 Hz, arom.), 7.16 (t, 2H, J = 8.0 Hz, arom.), 7.24 (d, 1H, J = 7.2 Hz, arom.), 7.50 (s, 1H, CH), 7.51 (s, 1H, NH), 7.97 (s, 2H, NH), 10.60 (s, 1H, NH). ^{13}C NMR

(100 MHz, DMSO-d_6): 37.5 (CH_2), 113.5, 116.5, 121.2, 125.2, 125.7, 127.9, 129.6, 130.1, 130.8, 137.6, 143.3 (=CH), 154.6 (C = O), 164.2 (C = O), 170.2 (C = O). Anal. Calcd for $C_{18}H_{14}Cl_2N_4O_3S$: C, 49.41; H, 3.23; N, 12.81. Found: C, 49.48; H, 3.29; N, 12.72. ESI-MS m/z 436/438 $(M + H)^+$.

[2-(2,6-Dichloro-phenylamino)-phenyl]-acetic acid N-(4-oxo-2-thioxo-thiazolidin-5-ylidenemethyl)-hydrazide (**3.3**). Yield 80%, gray powder, mp 193–194 °C (AcOH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.70 (s, 2H, CH_2), 6.32 (d, 1H, $J = 7.9$ Hz, arom.), 6.89 (t, 1H, $J = 7.4$ Hz, arom.), 7.05–7.10 (m, 2H, arom.), 7.17 (t, 1H, $J = 7.9$ Hz, arom.), 7.25 (t, 1H, $J = 7.4$ Hz, arom.), 7.50–7.53 (m, 3H, CH, NH, arom.), 9.87 (s, 1H, NH), 10.92 (s, 1H, NH), 12.93 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 37.5 (CH_2), 116.7, 121.3, 124.4, 125.8, 128.2, 129.6, 130.0, 131.2, 137.5, 143.3, 145.4 (=CH), 152.4 (C = O), 166.4 (C = O), 171.9 (C = O). Anal. Calcd for $C_{18}H_{14}Cl_2N_4O_4S_2$: C, 47.69; H, 3.11; N, 12.36. Found: C, 47.58; H, 3.20; N, 12.42. ESI-MS m/z 452/454 $(M + H)^+$.

[2-(2,6-Dichloro-phenylamino)-phenyl]-acetic acid N-(3-ethyl-4-oxo-2-thioxo-thiazolidin-5-ylidenemethyl)-hydrazide (**3.4**). Yield 70%, gray powder, mp 212–213 °C (AcCN). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.09 (t, 3H, $J = 6.4$ Hz, CH_3), 3.72 (s, 2H, CH_2), 3.94 (q, 2H, $J = 6.4$, 13.4 Hz, CH_2), 6.31 (d, 1H, $J = 8.0$ Hz, arom.), 6.89 (d, 1H, $J = 7.3$ Hz, arom.), 7.07 (t, 1H, $J = 7.3$ Hz, arom.), 7.15 (t, 1H, $J = 8.0$ Hz, arom.), 7.25 (d, 1H, $J = 7.3$ Hz, arom.), 7.49–7.51 (m, 2H, arom.), 7.71 (m, NH, CH), 10.02 (s, 1H, NH), 10.99 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 12.4 (CH_3), 37.5 (CH_3), 39.1 (CH_2), 116.8, 121.3, 124.3, 125.8, 128.2, 129.6, 130.0, 131.2, 137.5, 143.3 (=CH), 156.0 (C = O), 164.7 (C = O), 171.9 (C = S). Anal. Calcd for $C_{19}H_{16}Cl_2N_4O_2S_2$: C, 48.83; H, 3.45; N, 11.99. Found: C, 48.73; H, 3.40; N, 11.92. ESI-MS m/z 480/482 $(M + H)^+$.

[2-(2,6-Dichloro-phenylamino)-phenyl]-acetic acid N-(4-oxo-2-thioxo-3-p-tolyl-thiazolidin-5-ylidenemethyl)-hydrazide (**3.5**). Yield 83%, orange powder, mp 156–157 °C (AcOH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.30 (s, 3H, CH_3), 3.75 (s, 2H, CH_2), 6.33 (d, 1H, $J = 7.6$ Hz, arom.), 6.90 (t, 1H, $J = 7.6$ Hz, arom.), 7.06–7.11 (m, 3H, arom.), 7.16 (d, 1H, $J = 8.0$ Hz, arom.), 7.19–7.30 (m, 3H, arom.), 7.52 (d, 2H, $J = 8.0$ Hz, arom.), 7.75 (s, 1H, NH), 7.84 (s, 1H, CH), 10.08 (s, 1H, NH), 11.05 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 21.4 (CH_3), 37.3 (CH_2), 116.7, 121.1, 123.0, 124.0, 125.2, 128.5, 129.6, 130.0, 130.1, 131.1, 133.2, 137.1, 141.4, 142.3 (=CH), 152.1 (C = O), 169.3 (C = O), 172.0 (C = S). Anal. Calcd for $C_{25}H_{20}Cl_2N_4O_4S_2$: C, 55.25; H, 3.71; N, 10.31. Found: C, 55.18; H, 3.39; N, 10.42. ESI-MS m/z 542/544 $(M + H)^+$.

[2-(2,6-Dichloro-phenylamino)-phenyl]-acetic acid N-[3-(4-fluorophenyl)-4-oxo-2-thioxo-thiazolidin-5-ylidenemethyl)-hydrazide (**3.6**). Yield 83%, yellow powder, mp 177–178 °C (AcOH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.76 (s, 2H, CH_2), 6.33 (d, 1H, $J = 8.4$ Hz, arom.), 6.90 (t, 1H, $J = 7.2$ Hz, arom.), 7.09 (t, 1H, $J = 7.2$ Hz, arom.), 7.17 (t, 1H, $J = 8.0$ Hz, arom.), 7.29 (d, 1H, $J = 8.4$ Hz, arom.), 7.32–7.34 (m, 4H, arom.), 7.52 (d, 2H, $J = 8.0$ Hz, arom.), 7.81 (s, 1H, NH), 7.82 (s, 1H, CH), 10.10 (s, 1H, NH), 11.08 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 37.7 (CH_2), 116.5, 122.1, 123.8, 124.3, 125.7, 128.4, 129.8 (d, $J = 30.0$ Hz, C–F), 130.9, 131.3, 133.1, 137.6, 141.2, 143.3 (=CH), 152.4 (C = O), 162.8 (d, $J = 300.0$ Hz, C–F), 169.4 (C = O), 170.5 (C = S). Anal. Calcd for $C_{24}H_{17}Cl_2FN_4O_2S_2$: C, 52.66; H, 3.13; N, 10.23. Found: C, 52.72; H, 3.19; N, 10.32. ESI-MS m/z 546/548 $(M + H)^+$.

[2-(2,6-Dichloro-phenylamino)-phenyl]-acetic acid N-(4-oxo-2-thioxo-3-(3-trifluoromethylphenyl)thiazolidin-5-ylidene-methyl)-hydrazide (**3.7**). Yield 77%, yellow powder, mp 208–209 °C (AcOH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.76 (s, 2H, CH_2), 6.33 (d, 1H, $J = 7.4$ Hz, arom.), 6.90 (t, 1H, $J = 7.4$ Hz, arom.), 7.08 (t, 1H, $J = 6.8$ Hz, arom.), 7.17 (t, 1H, $J = 7.8$ Hz, arom.), 7.29 (d, 1H, $J = 6.8$ Hz, arom.), 7.51 (s, 1H, CH), 7.52 (s, 1H, NH), 7.62 (d, 1H, $J = 7.4$ Hz, arom.), 7.75–7.76 (m, 4H, arom.), 7.83 (d, 2H, $J = 7.8$ Hz, arom.), 10.09 (s, 1H, NH), 11.03 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 36.2 (CH_2), 117.1, 121.7, 129.7, 131.2, 133.2, 134.1, 136.7, 137.5, 139.0, 139.4, 140.4, 140.9, 141.7, 143.1, 147.0 (=CH), 152.3 (C = O), 166.5 (C = O), 178.7 (C = S). Anal. Calcd for $C_{25}H_{17}Cl_2F_3N_4O_2S_2$: C, 50.26; H, 2.87; N, 9.38. Found: C, 50.32; H, 2.79; N, 9.32. ESI-MS m/z 596/598 $(M + H)^+$.

N-{4-[5-(N-{2-[2-(2,6-Dichloro-phenylamino)-phenyl]-acetyl}-hydrazinomethylene)-4-oxo-2-thioxo-thiazolidin-3-yl]-phenyl}-acetamide (**3.8**). Yield 80%, orange powder, mp 190–191 °C (DMF:EtOH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.07 (s, 3H, CH_3), 3.75 (s, 2H, CH_2), 6.33 (d, 1H, $J = 8.0$ Hz, arom.), 6.90 (t, 1H, $J = 7.6$ Hz, arom.), 7.09 (t, 1H, $J = 8.0$ Hz, arom.), 7.17–7.19 (m, 3H, arom.), 7.28 (d, 1H, $J = 7.6$ Hz, arom.), 7.51 (s, 1H, CH), 7.53 (s, 1H, NH), 7.66 (d, 2H, $J = 8.8$ Hz, arom.), 7.76–7.80 (m, 2H, arom.), 10.07 (s, 1H, NH), 11.06 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 21.4 (CH_3), 37.4 (CH_2), 117.8, 121.8, 123.6, 124.8, 125.9, 128.5, 129.9, 130.1, 130.5, 131.6, 133.8, 137.6, 141.4, 143.5 (=CH), 151.5 (C = O), 160.9 (C = O), 164.7 (C = O), 171.6 (C = S). Anal. Calcd for $C_{26}H_{21}Cl_2N_5O_3S_2$: C, 53.24; H, 3.61; N, 11.94. Found: C, 53.32; H, 3.74; N, 11.82. ESI-MS m/z 585/587 $(M + H)^+$.

Acetic acid 3-[5-(N-{2-[2-(2,6-dichloro-phenylamino)-phenyl]-acethyl}-hydrazinomethylene)-4-oxo-2-thioxo-thiazolidin-3-yl]-phenyl ester (**3.9**). Yield 88%, orange powder, mp 151–152 °C (AcOH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.29 (s, 3H, CH_3), 3.76 (s, 2H, CH_2), 6.39 (d, 1H, $J = 7.6$ Hz, arom.), 6.91 (t, 1H, $J = 7.1$ Hz, arom.), 7.09 (t, 1H, $J = 7.1$ Hz, arom.), 7.17 (t, 1H, $J = 8.0$ Hz, arom.), 7.25–7.30 (m, 5H, arom.), 7.52 (d, 2H, $J = 8.0$ Hz, arom.), 7.76–7.78 (m, 2H, CH. NH), 10.07 (s, 1H, NH), 11.05 (s, 1H, NH). ^{13}C NMR(100 MHz, DMSO-d_6): 21.4 (CH_3), 37.0 (CH_2), 116.0, 121.1, 123.4, 124.4, 125.1, 128.3, 129.6, 130.3, 130.6, 131.4, 133.9, 137.6, 143.3 (=CH), 151.4 (C = O), 169.1 (C = O), 169.5 (C = O), 172.0 (C = S). Anal. Calcd for $C_{26}H_{20}Cl_2N_4O_4S_2$: C, 53.16; H, 3.43; N, 9.54. Found: C, 53.22; H, 3.50; N, 9.60. ESI-MS m/z 586/588 $(M + H)^+$.

Pharmacology

In vitro anticancer assay

Anticancer *in vitro* assay was performed on a panel of approximately 60 human tumor cell lines derived from nine

neoplastic diseases, in accordance with a protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda.[29–31] Tested compounds were added to the culture at a single concentration (10^{-5} M), and the cultures were incubated for 48 h. End point determinations were made with a protein binding dye, sulforhodamine B. Results for each tested compound were reported as the GP% of the treated cells when compared with the untreated control cells. GP% was evaluated spectrophotometrically versus controls not treated with test agents.

Antitrypanosomal activity assay

Bloodstream forms of *Tbb* strain 90–13 were cultured in HMI9 medium supplemented with 10% FCS at 37 °C under an atmosphere of 5% CO_2.[34] In all experiments, log-phase parasite cultures were harvested by centrifugation at 3000 g and immediately used. Drug assays were based on the conversion of a redox-sensitive dye (resazurin) to a fluorescent product by viable cells as previously described.[35] Drug stock solutions were prepared in pure DMSO. *Trypanosoma brucei* bloodstream forms (10^5 cells/mL) were cultured in 96-well plates either in the absence or in the presence of different concentrations of inhibitors in a final volume of 200 µL. After an 72-h incubation, resazurin solution was added in each well at the final con centration of 45 µM and fluorescence was measured at 530- and 590-nm absorbance after a further 4-h incubation. The percentage of inhibition of parasite growth rate was calculated by comparing the fluorescence of parasites maintained in the presence of drug to that in the absence of drug. DMSO was used as control. Concentration inhibiting 50% of parasite growth (IC_{50}) was determined from the dose-response curve with a drug concentrations ranging from 10 to 0.625 µg/mL and presented in µM. IC_{50} value is the mean ± the standard deviation of three independent experiments.

Anti-inflammatory activity in vivo

The anti-inflammatory (antiexudative) activity of test compounds was evaluated using the carrageenan-induced rat paw edema method.[32,33] For antiexudative tests, adult rats of either sex weighing 190–220 g were used. Diclofenac sodium (8 mg/kg) and ketorolac tromethamine (10 mg/kg) were used as reference compounds. All animals were fed standard laboratory chow and tap water before the experiments. The standard drug and the test compounds (50 mg/kg body weight) were dissolved in saline solution with one drop of Tween 80™ and intraperitoneally injected. Control rats received only saline solution with one drop of Tween 80™; 40 min later, 0.1 mL of 2% carrageenan solution in saline was injected in the subplantar region of the right hind paw of each rat. The hind paw volume (in mL) was measured with an electronic onkograph immediately before and 4 h after carrageenan injection, and paw edema was compared with control group. The anti-inflammatory activity was expressed as a decrease in rat paw edema and is given in percentage. The procedure employed for anti-inflammatory evaluation was reviewed and approved by the Danylo Halytsky Lviv National Medical University Animal Ethical Committee (protocol № 3 from 18/03/2013).

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