Synthesis of 4,5-Dihydro-1H-[1,2]dithiolo[3,4-c]quinoline-1-thione Derivatives and Their Application as Protein Kinase Inhibitors

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Abstract: This study represents the design and synthesis of a new set of hybrid and chimeric derivatives of 4,5-dihydro-4,4-dimethyl-1H-[1,2]dithiolo[3,4-c]quinoline-1-thiones, the structure of which the tricyclic fragment linearly bound or/and condensed with another heterocyclic fragment. Using the PASS Online software, among the previously synthesized and new derivatives of 1,2-dithiolo[3,4-c]quinoline-1-thione we identified 12 substances with pleiotropic activity, including chemoprotective and antitumor activity. All the synthesized derivatives were screened for their inhibitory assessment against a number of kinases. Compounds which exhibited prominent inhibition percentage in cells (>85%) were also examined for their inhibitory efficiency on human kinases via ELISA utilizing sorafenib as a reference standard to estimate their IC50 values. It was revealed that compounds 2a, 2b, 2c, and 2q displayed a significant inhibition JAK3 (IC50 = 0.36 \( \mu \)M, 0.38 \( \mu \)M, 0.41 \( \mu \)M, and 0.46 \( \mu \)M, respectively); moreover, compounds 2a and 2b displayed excellent activities against NPM1-ALK (IC50 = 0.54 \( \mu \)M, 0.25 \( \mu \)M, respectively), against cRAF[Y340D][Y341D], compound 2c showed excellent activity, and compound 2q showed weak activity (IC50 = 0.78 \( \mu \)M, 5.34 \( \mu \)M, respectively) (sorafenib IC50 = 0.78 \( \mu \)M, 0.43 \( \mu \)M, 1.95 \( \mu \)M, respectively). Thus, new promising preferred structures for the creation of drugs for the treatment of cancer and other multifactorial diseases in the future have been found.

Keywords: 1,2-dithiol-3-thione; 2,2-disubstituted-1,2-dihydroquinolines; dithioloquinolinethiones; non-selective anti-kinase activity; bioactivity profiles prediction in silico; PASS online

1. Introduction

Interest in 3H-1,2-dithiol-3-thione (DTT) derivatives has developed continuously but unevenly for nearly 140 years. For the first time, a compound containing a 1,2-dithiol-3-thione ring (4,5-dimethyl-1,2-dithiol-3-thione I, Figure 1) was obtained in 1884 by Barbaglia [1]. The study of the chemistry of these five-membered pseudoaromatic compounds started to develop later, in the middle of the 20th century, after the appearance of a number of studies devoted to the synthesis of DTT derivatives and their isolation from cruciferous plants [2–7].

In the mid-1980s, there was a surge in interest in DTT derivatives after the introduction of Oltipraz (4-methyl-5-(2-pyrazinyl)-1,2-dithiol-3-thione II, Figure 1) into clinical practice as an antiparasitic agent against Schistosoma mansoni (1985, pharmaceutical company Aventis) [8]. Further studies have shown that oltipraz also exhibits cancer-preventive activity and can be used in the treatment of cancer [9–12]. Oltipraz and its analogues exhibit a cancer-preventive effect via the induction of cell cytoprotective enzymes involved in the detoxification of carcinogens and via stimulation of the restoration of DNA damaged by carcinogens [13,14], or by the activation of transcription factors [15,16].
3H-1,2-Dithiol-3-thiones inhibit the action of the resulting hydrogen peroxide, protect mitochondria from oxidative stress, and increase their antioxidant potential [17,18]. The 1,2-dithiol-3-thione fragment is an effective endogenous donor of hydrogen sulfide, an important gaseous signaling molecule (gas transmitter) in the human body, and is involved in the regulation of a number of physiological and pathophysiological processes [19,20]. One of the worldwide most-studied hydrogen sulfide donors is 5-(4-hydroxyphenyl)-3H-1,2-dithiole-3-thione ((ADT-OH) III, Figure 1), which inhibits apoptosis and removes ROS through stimulation of glutathione and glutathione-S-transferase [20].

DTT, as H₂S-releasing compounds, not only exhibit anticancer properties, but also analgesic activity [21], and protect cardiomyocytes from ischemic cell death [22]. Hybrids of DTT and non-steroidal anti-inflammatory drugs (aspirin, diclofenac) are being developed as promising drugs for the treatment of inflammatory diseases of various etiologies [23,24]. The presence of fragments with different pharmacotherapeutic profiles in one molecule can be useful for a reduction in side effects, enhancement of the action of a drug or reduction in resistance to it, and also for expanding the range of its application [25,26].

Therefore, the design of hybrid or chimeric compounds in the structure of which the dithiol ring is combined with another heterocyclic fragment (for example, azaheterocycle) with a certain pharmacological activity is of current interest. Among azaheterocycles, 2,2-disubstituted-1,2-dihydroquinolines functionalized at the benzene ring and/or the nitrogen atom (DHQ), which have a wide spectrum of biological activity—anticoagulant, antimalarial, antiparasitic, antibacterial, antidiabetic, anti-inflammatory, neuroprotective, and hepatoprotective activity—are of particular interest [27–33].

For some representatives of hybrid 4,5-dihydro-4,4-dimethyl-1H-[1,2]dithiolo[3,4-c]quinoline-1-thiones (DTT-DHQ), in which dithiolthione and hydroquinoline cycles are annelated via the [c] bond of the latter (Figure 2), we have previously revealed an inhibitory effect on the blood coagulation system [34]. We also studied the antimicrobial antifungal activity of these polycyclic dithiolquinolinethiones and identified compounds with an activity exceeding the effects of ampicillin and streptomycin. Their antifungal activity was higher than that of the reference drugs ketoconazole and bifonazole [35]. Recently, using the PASS Online (Prediction of Activity Spectra for Substances) software [36], we determined the probable anti-inflammatory effect of various dithiolquinolinethione derivatives and experimentally confirmed that the anti-inflammatory activity of the studied compounds is comparable to or higher than that of the reference drug indomethacin [37] (Figure 2).

Due to the discovered multiple (pleiotropic) action of annelated DTT, the search for new types of activity, in particular, anti-cancer activity, in previously obtained dithiolquinolinethione derivatives, the targeted synthesis and study of the activity of new representatives of this series are of considerable interest for medicinal chemistry and pharmaceutical science.
It is known that the causes of cancer, diabetes, inflammatory processes, and other multifactorial diseases are mutation processes and/or activation of enzymes of the protein kinase family [38,39]. The human genome contains 518 kinases that transfer the γ-phosphate of ATP to the hydroxyl group of tyrosine, serine, or threonine residues of the substrate. Protein kinases play a key role in cell proliferation, metabolism, and apoptosis; therefore, they have become a target for anticancer drug therapy [40]. One of the strategies of antitumor therapy is the inhibition of protein kinases by low-molecular-weight ATP mimetic compounds which block oncogene-induced cell signaling pathways, affect the proteins that regulate gene functions, induce the apoptosis of cancer cells, etc. [41].

Most of the early kinase inhibitors exhibited poor selectivity for undesirable targets such as ion channels, cytochrome P450 (CYP), and other proteins, causing side effects [42,43]. In order to minimize the risk of side effects, a current trend is the development of targeted (highly selective) kinase inhibitors with directed pathogenetic action [44]. However, a relatively small number of selective kinase inhibitors have been approved in practice, since the clinical use of these inhibitors has led to the emergence of drug-resistant tumors. In many patients, the response to small molecule kinase inhibitors was accompanied by tumor recurrence, making these inhibitors less effective than expected [45–48]. This resistance was associated with a number of mechanisms that include the gene amplification of oncogenic kinase [49] and alternative signaling pathways or signaling plasticity [50].

The insensitivity of the drug to kinase mutations is the main task of molecular design and the synthesis of targeted antitumor organic compounds. Changing the selectivity profile in the treatment of complex diseases such as cancer can lead to an improvement in the therapeutic quality of the compound. In the last decade, studies and clinical practice for drugs have established the advantages of multi-inhibition in cancer therapy in comparison with monotarget inhibition. In antitumor therapy, multikinase inhibition may be useful primarily for the inhibition of rapidly mutating kinases [51–56]. Multitarget drug discovery (MTDDa) drugs that affect more than one target link can provide super-efficacy and safety comparable to monotargeted drugs [57,58].

The aim of this study was molecular modeling, computer screening for potential antitumor activity, synthesis of linearly bound (hybrid molecules) and condensed derivatives (chimeric molecules) of 4,5-dihydro-1H-[1,2]dithiolo[3,4-c]quinoline-1-thiones, and the investigation of their inhibitory activity against a number of protein kinases, namely, NPM1-ALK, ALK, EGFR[L858R][T790], cRAF[Y340D][Y341D], JAK2, and JAK3.

Using the PASS Online software, among the previously synthesized and new hybrid and chimeric derivatives of 1,2-dithiolo[3,4-c]quinoline-1-thione we identified 12 substances with pleiotropic activity, including chemoprotective and antitumor activity, and experimentally confirmed their inhibitory activity. For the leading compounds (phenylpiperazinylcarbonythiyl- and 8-morpholinylcarbonythiyl-derivatives of 1,2-dithiolo[3,4-c]quinoline-1-thiones, substituted imino derivative of 1,2-dithiolo[3,4-c]pyrrolo[3,2,1-ij]quinoline), IC₅₀ concentrations (µM) were calculated. The noted high activity of the last compound, which based on in silico predictions is inactive, indicates the novelty of the structure of this molecule in relation to known drugs.
2. Results and Discussion
2.1. Chemistry

The strategy for the molecular design of DTT-DHQ derivatives was the diversification of the tricyclic structure by introducing various substituents in the aromatic ring and/or to the nitrogen atom of the quinoline fragment, annealing it to five- or six-membered heterocycles, and also the substitution of the exo-sulfur atom. At the same time, by combining a dithiolooquinolinethione fragment with some pharmacophores (dioxane, pyrrole) by condensation via the common bond, new chimeric molecules were constructed. The new linearly linked hybrid molecules were created by linking the original ligand with various pharmacophore ligands via a metabolizable linker (with piperidine, morpholine, piperazinone, pyrrolidine, isoindole, thiophene, benzathine, etc.). In addition, a hybrid chimeric structure containing both annulated and linearly linked ligands was assembled (Figure 3).

![Figure 3. Structure of hybrid and/or chimeric 4,5-Dihydro-4,4-dimethyl-1H-[1,2]dithiolo[3,4-c]quinoline-1-thiones.](image)

General routes for the synthesis of target compounds and some intermediates are shown in Scheme 1. Initial 2,2,4-trimethyl-1,2-dihydroquinolines 1a–h substituted at the aromatic ring or nitrogen atom were obtained according to the known methods [59–61]. 8-Heterylcarbonothionyl-DTT-DHQ 2a–d were obtained from the reaction of N-alkyldihydroquinoline-6-carbaldehydes 1g,h of cyclic secondary amines and excess of elemental sulfur using the previously developed method [62]. Intermediate dithiolo[3,4-c]quinoline-1-thiones 2e–g and previously undescribed tetracyclic DTT 2h were synthetized by sulfurisation of dihydroquinoline 1a–d when refluxed in dimethylformamide with a five-fold excess of sulfur, according to a previously described procedure [63] (Scheme 1). Further synthesis of all dithiolothiones 2e–h was carried out according to the methods developed by us earlier [3–7], based on the action of electrophilic reagents on hydrogen atoms in position 6 and/or the secondary amino group of the dihydroquinoline cycle, as well as on the thiocarbonyl group (Scheme 1). Therein, previously undescribed DTT derivatives were synthetized. N-acyl-[1,2]dithiolo[3,4-c]quinoline-1-thiones 2i–m were obtained by the reaction of dithiolothiones 2f–h with various carbonyl chlorides by reflux in toluene [64,65]. Annellation of the pyrrole-1,2-dione fragment by the Stolle reaction was carried out by the action of oxalyl chloride on dithiolothione 2h by reflux in dry toluene, and pyrrolo[3,2,1-i][quinoline-1,2-dione 2n was synthetized [66]. Arylamino derivative 2p
was obtained by sequential reactions of alkylation of DTT-DHQ \(2e\) with methyl iodide and condensation of the resulting iodomethylate \(2o\) with arylamine [67]. Annulated dithiolo[3,4-c]pyrrolo[3,2,1-ij]quinoline-4,5-dione \(2q\) was obtained by the acylation of the latter with oxalyl chloride [67].

The structures of the new synthesized compounds \(2h, 2i–n,\) and \(2q\) were unambiguously confirmed by \(^1\)H and \(^{13}\)C NMR spectroscopy data and HPLC–HRMS spectrometry. In the \(^1\)H NMR spectra of all target compounds \(2\), proton signals of heme-dimethyl groups were observed in the corresponding fields—at 1.4–2.5 ppm, C(9)-H proton signals shifted in a weak field to 8.6–9.5 ppm. Due to the anisotropic effect of the thioketone group (for compounds \(2p, q\)—arylimino group), the signals of other protons of the quinoline ring appear in the aromatic region of the spectrum [66,67].

| No  | R   | R’  | R”  |
|-----|-----|-----|-----|
| 1a  | H   | H   | H   |
| 1b  | CH₃O| H   | H   |
| 1c  | H   | CH₃O| H   |
| 1d  | O–CH₂–CH₂–O| H   |
| 1e  | H   | H   | CH₃ |
| 1f  | H   | H   | CH₃CH₃ |
| 1g  | -   | -   | CH₃OH |
| 1h  | -   | -   | CH₃CH₃ |

Scheme 1. Cont.
were observed. Peaks of protonated molecular ions, consistent with the structure of these compounds, were observed.

Previously confirmed by 1H and 13C NMR spectroscopy data and HPLC–HRMS spectrometry.

The signal for the carbon atom of the imino group at 166.6 ppm was detected in the spectrum.

In the 1H NMR spectra of all target compounds were observed in the corresponding fields—a t 1.4–2.5 ppm, C(9)-H proton signals shifted appear in the aromatic region of the spectrum [66,67].

In the mass spectra of compounds –i, –n, and –q, peaks of protonated molecular ions, consistent with the structure of these compounds, were observed.

| №  | X   | R       | R'     | R''    |
|-----|------|---------|--------|--------|
| 2a  | O    | -       | -      | CH3    |
| 2b  | NC6H5 | -     | -      | CH3    |
| 2c  | O    | -       | -      | CH2CH3 |
| 2d  | CH2  | -       | -      | CH2CH3 |
| 2e  | -    | H       | H      | -      |
| 2f  | -    | CH3O    | H      | -      |
| 2g  | -    | H       | CH3O   | -      |
| 2h  | -    | O-CH2-CH2-O | -   |
| 2i  | -    | CH3O    | H      | -      |
| 2j  | -    | CH3O    | H      | -      |
| 2k  | -    | CH3O    | H      | -      |
| 2l  | -    | H       | CH3O   | -      |
| 2m  | -    | O-CH2-CH2-O | -   |

Scheme 1. Synthesis of the key intermediates (1g,h, 2e–h, 2o, and 2p) and target compounds (2a–d, 2h, 2i–n, and 2q). Reagents and conditions: (a) PCl5, DMF, 0 °C, 0.5 h, 80 °C, 3 h; (b) secondary heterocyclic amine, elemental sulfur, DMF, reflux, 15 h; (c) elemental sulfur, DMF, reflux, 15 h; (d) R-carbonyl chloride, dry toluene, reflux, 8 h; (e) oxalyl chloride, dry toluene, reflux, 2 h; (f) CH3I, (CH3)2CO, reflux, 2 h; (g) m-anisidine, dry i-PrOH, pyridine, reflux, 3 h; (h) oxalyl chloride, dry toluene, reflux, 1.5 h.

The signal of the NH proton of the hydroquinoline fragment of compound 2h was revealed in the characteristic region of 6.0 ppm. Compared to the spectra of the original dithiolothiones 2f–h, in the spectra of compounds 2i–n, no NH proton signal was detected, and in the part of the spectrum corresponding to the aromatic protons of the compound 2n, one fewer proton was observed. Signals of corresponding N-acyl fragments appeared in characteristic regions in the spectra of compounds 2i–m [64,65]. In addition, in the spectra of compounds 2j,k, signals of two hydrogen atoms of the substituted acetyl fragment were observed in the form of broadened singlets in the characteristic region of 3.8–4.1 ppm [65].

Good-quality spectra of compound 2n were not obtained due to its low solubility in DMSO. Characteristic signals of the carbon atom of the thiocarbonyl group in the region of 210.7–211.1 ppm were revealed in the 13C NMR spectra of compounds 2h and 2i–m. The signal for the carbon atom of the imino group at 166.6 ppm was detected in the spectrum of the arylimino derivative 2q. In the mass spectra of compounds 2h, 2i–n, and 2q, peaks of protonated molecular ions, consistent with the structure of these compounds, were observed.

2.2. Biological Activity Profile Evaluation by PASS

The computer prediction of the biological activity of the simulated compounds was carried out using the PASS Online web resource [36]. PASS Online predicts more than 4300 types of biological activity based on the analysis of a training set containing information on more than 300,000 drug substances and biologically active compounds, with an average accuracy of 95%. The predicted PASS spectrum of biological activity of an organic compound includes pharmacological effects, molecular mechanisms of action, specific toxicity and side effects, metabolism, as well as their effects on undesirable targets,
molecular transport, and gene expression. Since PASS allows simultaneous prediction of the interaction of chemical compounds with a large number of biological targets, substances with a pleiotropic effect can be selected based on the results of the prediction. According to the results of the prediction of biological activity using the PASS Online program, almost all dithioloquinolinethiones 2 have multiple activities and, in addition to anti-inflammatory and anti-schistosomal actions, with a probability of more than 50% can possess chemoprotective (58–72%) and antitumor effects (56–66%) and be apoptosis agonists (59–81%) (Table 1). In addition, compounds 2a–d, with a probability of 58–61%, can be dual-specific phosphatase inhibitors. Out of all compounds tested, only for derivative 2q, containing an arylimino group in the dithiol fragment, the predicted spectrum of activity was very narrow and included only the inhibition of gluconate-2-dehydrogenase (58%).
Table 1. Structural formulae of the studied compounds and the results of in silico predictions.

| N   | Structure | Inflammatory Bowel Disease Treatment Pa/Pi | Antischistosomal Drug Treatment Pa/Pi | Anti-inflammatory Drug Treatment Pa/Pi | Chemoprotective Treatment Pa/Pi | Maillard Reaction Inhibitor Pa/Pi | Dual Specificity Phosphatase Inhibitor Pa/Pi | Hepatic Disorders Treatment Pa/Pi | Apoptosis Agonist Pa/Pi | Other Activity (non-small cell lung cancer) Pa/Pi |
|-----|-----------|-------------------------------------------|-------------------------------------|--------------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|----------------------|-------------------------------------------------|
| 2a  | ![Molecule](image) | 0.925 / 0.003                           | 0.819 / 0.001                        | 0.819 / 0.005                        | 0.635 / 0.004                   | 0.616 / 0.004                   | 0.611 / 0.003                   | -                               | 0.615 / 0.025 | Growth stimulant 0.748 / 0.003 |
| 2b  | ![Molecule](image) | 0.924 / 0.003                           | 0.828 / 0.001                        | 0.798 / 0.007                        | 0.621 / 0.004                   | 0.620 / 0.003                   | 0.605 / 0.003                   | 0.589 / 0.007 | 0.600 / 0.026 | Growth stimulant 0.710 / 0.003 |
| 2c  | ![Molecule](image) | 0.924 / 0.003                           | 0.811 / 0.001                        | 0.804 / 0.006                        | 0.616 / 0.004                   | 0.612 / 0.004                   | 0.593 / 0.003                   | 0.572 / 0.008 | 0.573 / 0.029 | Growth stimulant 0.715 / 0.003 |
| 2d  | ![Molecule](image) | 0.924 / 0.003                           | 0.818 / 0.001                        | 0.795 / 0.007                        | 0.625 / 0.004                   | 0.617 / 0.004                   | 0.581 / 0.003                   | 0.579 / 0.007 | 0.590 / 0.028 | Growth stimulant 0.705 / 0.003 |
| 2h  | ![Molecule](image) | 0.948 / 0.002                           | 0.861 / 0.001                        | 0.922 / 0.004                        | 0.717 / 0.003                   | 0.704 / 0.002                   | -                              | 0.805 / 0.004 | -                              |                                                      |
| 2i  | ![Molecule](image) | 0.931 / 0.003                           | 0.806 / 0.001                        | 0.895 / 0.004                        | 0.614 / 0.004                   | 0.619 / 0.004                   | -                              | 0.654 / 0.005 | 0.706 / 0.014 | Antineoplastic 0.556 / 0.005 |

**Note:** The table includes structural formulae for the studied compounds along with their predicted activities. The values represent the Pa/Pi ratios, with higher values indicating better activity. The activities include Anti-inflammatory, Antischistosomal, Chemoprotective, Maillard Reaction, Dual Specificity Phosphatase Inhibitor, Hepatic Disorders Treatment, Apoptosis Agonist, and Other Activity (non-small cell lung cancer). The table shows a variety of compounds with different activities, highlighting the potential therapeutic applications of these molecules.
Table 1. Cont.

| N  | Structure | Inflammatory Bowel Disease Treatment Pa/Pi | Antischistosomal Pa/Pi | Anti-inflammatory Pa/Pi | Chemoprotective Pa/Pi | Maillard Reaction Inhibitor Pa/Pi | Dual Specificity Phosphatase Inhibitor Pa/Pi | Hepatic Disorders Treatment Pa/Pi | Apoptosis Agonist Pa/Pi | Other Activity Pa/Pi |
|----|-----------|------------------------------------------|------------------------|------------------------|-----------------------|-------------------------------|-------------------------------------------|----------------------------|------------------|-------------------|
| 2j | ![Structure](image1.png) | 0.918/0.003 | 0.807/0.001 | 0.852/0.005 | 0.622/0.004 | - | - | - | 0.811/0.007 | Antineoplastic (non-small cell lung cancer) 0.660/0.004 Anaphylatoxin receptor antagonist 0.642/0.032 |
| 2k | ![Structure](image2.png) | 0.905/0.004 | 0.790/0.001 | 0.805/0.006 | 0.598/0.005 | 0.575/0.004 | - | - | 0.760/0.010 | Antineoplastic (non-small cell lung cancer) 0.572/0.005 |
| 2l | ![Structure](image3.png) | 0.918/0.003 | 0.802/0.001 | 0.821/0.005 | 0.579/0.005 | 0.602/0.004 | - | - | 0.633/0.023 | |
| 2m | ![Structure](image4.png) | 0.931/0.003 | 0.794/0.001 | 0.872/0.005 | 0.604/0.005 | 0.587/0.004 | - | 0.667/0.005 | 0.676/0.017 | - |
| N  | Structure | Inflammatory Bowel Disease Treatment Pa/Pi | Antischistosomal Pa/Pi | Anti-inflammatory Pa/Pi | Chemoprotective Pa/Pi | Maillard Reaction Inhibitor Pa/Pi | Dual Specificity Phosphatase Inhibitor Pa/Pi | Hepatic Disorders Treatment Pa/Pi | Apoptosis Agonist Pa/Pi | Other Activity Pa/Pi |
|----|-----------|------------------------------------------|------------------------|------------------------|-----------------------|-------------------------------|---------------------------------|-----------------------------|--------------------------|---------------------|
| 2n | ![Structure 2n](image) | 0.932/0.003 | 0.830/0.001 | 0.877/0.005 | 0.652/0.004 | 0.656/0.003 | - | 0.827/0.004 | - | - |

(-) Activity < 0.5.

Gluconate 2-dehydrogenase (acceptor) inhibitor
0.581/0.137
Amloid beta precursor protein antagonist
0.498/0.005
2.3. Biological Evaluation

For the obtained DTT-DHQ derivatives 2a–d, 2h, 2i–n, and 2q, primary in vitro screening was carried out for the identification of the leading compounds and determination of their relative inhibitory activity against a number of protein kinases, NPM1-ALK, ALK, EGFR[L858R][T790], cRAF[Y340D][Y341D], JAK2, and JAK3, by ELISA. For the leading compounds, IC$_{50}$ concentrations (µM) were calculated (Table 2). Out of the 12 compounds tested, two compounds, 2k, m, demonstrated very low activity. For most of the chimeric molecules 2h,j,n, relative inhibitory activity against the studied protein kinases was moderate (5–56%). In relation to the studied protein kinase hybrid molecules 2a–d,i,l, they demonstrated inhibitory activity from moderate (12–81%) to high (84–96%) levels, while compounds 2a–c exhibited the maximum non-specific inhibitory effect (IC$_{50}$ 0.25–0.78 µM). The compound 2q, containing both hybrid and chimeric ligands, showed high activity against kinases JAK3 (99%, IC$_{50}$ 0.46 µM) and cRAF[Y340D][Y341D] (92%, IC$_{50}$ 5.34 µM).

Table 2. Inhibitory activity of compounds 2a–d, 2h, 2i–n, and 2q, and IC$_{50}$ (µM) of compounds 2a–c, 2q against protein kinases.

| N | NPM1-ALK | ALK | EGFR [L858R][T790] | CRAF [Y340D][Y341D] | JAK2 | JAK3 | CRAF [Y340D][Y341D] | JAK3 | NPM1-ALK |
|---|---------|-----|------------------|-------------------|------|------|-------------------|------|----------|
| 2a | 87 | 84 | 82 | 93 | 81 | 96 | 6.57 | 0.36 | 0.54 |
| 2b | 90 | 86 | 84 | 92 | 83 | 95 | N/A | 0.38 | 0.25 |
| 2c | 75 | 77 | 88 | 93 | 76 | 95 | 0.78 | 0.41 | - |
| 2d | 41 | 39 | 12 | 61 | 33 | 69 | - | - | - |
| 2h | 42 | 49 | 27 | 44 | 10 | 36 | - | - | - |
| 2i | 77 | 81 | 24 | 76 | 64 | 89 | - | - | - |
| 2j | 56 | 7 | 9 | 5 | 11 | 39 | - | - | - |
| 2k | 27 | 18 | -2 | 41 | 3 | 26 | - | - | - |
| 2l | 67 | 75 | 24 | 81 | 53 | 86 | - | - | - |
| 2m | 6 | -4 | 10 | 35 | 1 | 23 | - | - | - |
| 2n | 24 | 19 | 5 | 13 | 47 | 52 | - | - | - |
| 2q | 36 | 51 | 71 | 92 | 9 | 99 | 5.34 | 0.46 | - |
| sorafenib | - | - | - | - | - | - | 1.95 | 0.78 | 0.43 |

* The decrease in intensity compared to the fluorescence intensity for the native enzyme in a blank experiment without the addition of an inhibitor.

Thus, as a result of testing for the inhibition of protein kinases, substances were identified that exhibit a non-specific inhibitory effect at the nanomolar level against protein kinases JAK3 (IC$_{50}$ 0.36–0.46 µM), NPM1-ALK (IC$_{50}$ 0.25–0.54 µM), and cRAF[Y340D][Y341D] (IC$_{50}$ 0.78 µM) and providing promise for further research.

The highest activity was shown by hybrid derivatives of hydroquinoline dithiolothiones containing a thiocarbamamide group with a morpholine or piperazine residue in the aromatic ring, as well as a hybrid chimeric structure with a pyrroledione fragment fused at the «i and j» bonds of quinoline and an arylylidene fragment in the dithiol cycle.

3. Materials and Methods

3.1. Synthesis

NMR $^1$H and $^{13}$C spectra were registered on a Bruker DRX–500 (500.13 MHz and 125.76 MHz, respectively) spectrometer (Bruker Corporation, Billerica, MA, USA) in DMSO-d$_6$, and the internal standard was TMS. Melting points were determined on a Stuart SMP 30 (Cole-Palmer, Staffordshire, UK). To control the reagent and product individually, qualitative analysis of reaction mass was performed by TLC on a Merck TLC Silicagel 60 F254 chromatographic plate (Merck KGaA, Darmstadt, Germany). Eluents included methanol, chloroform, and their mixtures in various rations. The chromatograms were developed by the UV irradiator of chromatographic plates UFS 254/365 Sorbil (Production
company Imid, Krasnodar, Russia) and iodine vapor. Product purity was monitored by high-performance liquid chromatography with high-resolution mass spectrometric electrospray ionization detection (HPLCHRMS–ESI) in combination with UV detection. The analyses were performed on an Agilent 1260 Infinity chromatograph (Agilent Technologies, Santa Clara, CA, USA) and Agilent 6230 TOF LC/MS high-resolution time-of-flight mass detector. The ionization block was double electrospray; the signals were recorded in positive polarity; nebulizer N2 20 psig; desiccant gas N2, 6 mL/min; 325 °C; and mass detection range was 50–200 daltons. Capillary voltage 4.0 kV, fragmentor +191 V, skimmer +66 V, OctRF 750 V. A Poroshell 120 EC-C18 column (4.6 × 50 mm; 2.7 μm) was used. Gradient elution: acetonitrile/water (0.1% formic acid); flow rate 0.4 mL/min. Software for processing research results: MassHunter Workstation/Data Acquisition V.06.00 (Agilent Technologies, Santa Clara, CA, USA).

Commercially available reagents from Lancaster were also used in the syntheses. The starting compound 1a–f and intermediates 1g–h, 2e–g, 2o, p was synthesized according to a published method [59–61,63].

Target compounds 8-substituted 4,4,5-trimethyl-4,5-dihydro-1H-[1,2]dithio[3,4-c]quinoline-1-thiones 2a–2d were synthesized according to the procedure developed and described by us earlier [62].

General procedure for synthesis of substituted N-acyl-[1,2]dithiolo[3,4-c]quinoline-1-thione 2i–m: to a solution of the starting compounds 2f–h (5 mmol) in dry toluene (10 mL), a solution of corresponding acylchloride (5.5 mmol) in toluene (10 mL) was added dropwise under cooling. The reaction mixture was refluxed for 8–10 h while the reaction progress was monitored by TLC. Toluene was distilled off under reduced pressure, and the precipitate was filtered and recrystallized from toluene to furnish the desired products 2i–m.
Ar), 7.77 (d, J = 7.96 Hz, 1H, H-Ar), 7.72 (d, J = 4.2 Hz, 1H, H-4 Ar), 8.88 (s, 1H, H-9 quinoline); 13C NMR (DMSO-d6, 150 MHz) δ ppm: 25.1, 40.1, 55.3, 61.7, 108.1, 114.3, 124.2, 126.3, 127.6, 130.5, 133.0, 133.5, 133.9, 140.0, 155.6, 163.2, 179.6, 211.5; HPLC-HRMS (ESI) calc for C18H15N2O5S4 + H+, 406.0059; found, 406.0060 (see Supplementary Materials, Figures S8–S10).

1-[2-(8-Methoxy-4,4-dimethyl-1-thioxo-1,4-dihydro-5H-[1,2]dithiolo[3,4-c]quinolin-5-yl)-2-oxoethyl]pyrrolidin-2,5-dione (2i). Orange powder (yield 1.54 g, 71%), m.p. = 201–202 °C; 1H NMR (DMSO-d6, 500 MHz) δ ppm: 1.27 (br s, 3H, (CH3)2), 2.15 (br s, 3H, (CH3)2), 2.51 (s, 2H, CH2), 3.57 (s, 2H, CH2), 3.81 (s, 3H, CH3O), 4.07 (br s, 2H, CH2CO), 7.03 (d, J = 8.7 Hz, 1H, H-6(7) quinoline), 7.53 (d, J = 8.7 Hz, 1H, H-7(6) quinoline). 13C NMR (DMSO-d6, 125 MHz) δ ppm: 26.6, 27.1, 27.9, 42.4, 55.5, 61.6, 66.4, 108.7, 114.5, 126.9, 127.3, 127.8, 134.1, 157.3, 167.6, 176.7, 181.6, 211.1; HPLC-HRMS (ESI) calc for C16H18N2O5S3 + H+, 435.0503; found, 435.0502 (see Supplementary Materials, Figures S11–S13).

2-[2-(8-Methoxy-4,4-dimethyl-1-thioxo-1,4-dihydro-5H-[1,2]dithiolo[3,4-c]quinolin-5-yl)-2-oxoethyl]hexahydro-1H-isoinindle-1,3(2H)-dione (2k). Orange powder (yield 1.78 g, 73%), m.p. = 205–206 °C; 1H NMR (DMSO-d6, 500 MHz) δ ppm: 1.27–2.50 (m, 14H, 8H, 4 CH2), 2.84 (s, 2H, CH2), 3.81 (s, 3H, CH3O), 4.06 (br s, 2H, CH2CO), 7.04 (d, J = 8.5 Hz, 1H, H-6(7) quinoline), 7.59 (d, J = 8.5 Hz, 1H, H-7(6) quinoline), 8.79 (s, 1H, H-9 quinoline); 13C NMR (DMSO-d6, 125 MHz) δ ppm: 21.2, 23.0, 24.7, 25.9, 26.1, 34.9, 40.1, 41.9, 55.5, 51.6, 108.8, 114.4, 127.1, 127.5, 127.8, 134.1, 157.3, 167.8, 178.7, 181.6, 211.0; HPLC-HRMS (ESI) calc for C23H24N2O4S3 + H+, 489.0972; found, 489.0970 (see Supplementary Materials, Figures S14–S16).

5-[3-Chloro-1-benzothien-2-yl]carbonyl]-7-methoxy-4,4-dimethyl-4,5-dihydro-1H-[1,2]dithiolo[3,4-c]quinoline-1-thione (2l). Orange powder (yield 2.12 g, 87%), m.p. = 218–219 °C; 1H NMR (DMSO-d6, 500 MHz) δ ppm: 1.88 (br s, 6H, (CH3)2), 3.50 (s, 3H, CH3O), 6.48 (s, 1H, H-6 quinoline), 6.77 (d, J = 8.8 Hz, 1H, H-8 quinoline), 7.51 (t, J = 7.9 Hz, 1H, H-5(6) Ar), 7.54 (d, J = 7.9 Hz, 1H, H-6(5) Ar), 7.73 (d, J = 7.9 Hz, 1H, H-4(5) Ar), 8.02 (d, J = 7.9 Hz, 1H, H-7(4) Ar), 9.11 (d, J = 8.8 Hz, 1H, H-9 quinoline); 13C NMR (DMSO-d6, 125 MHz) δ ppm: 25.5, 55.2, 62.3, 110.2, 110.3, 117.5, 121.7, 122.8, 123.5, 123.9, 126.1, 128.0, 132.5, 134.6, 135.2, 137.3, 137.4, 158.8, 158.9, 161.9, 175.8, 211.1; HPLC-HRMS (ESI) calc for C22H16ClNO3S4 + H+, 489.9826; found, 489.9826 (see Supplementary Materials, Figures S17–S19).

5-(2-Fluorobenzoyl)-4,4-dimethyl-4,5,8,9-tetrahydro-1H-[1,4]dioxino[2,3-g][1,2]dithiolo[3,4-c]quinoline-1-thione (2m). Orange powder (yield 1.54 g, 69%), m.p. = 234–235 °C; 1H NMR (DMSO-d6, 500 MHz) δ ppm: 1.86 (br s, 6H, (CH3)2), 2.23 (s, 3H, CH3), 4.14 (d, J = 10.05 Hz, 4H, 2 CH2O), 6.34 (s, 1H, H-6 quinoline), 7.06 (t, J = 8.7 Hz, 1H, H-4(5) Ar), 7.17 (t, J = 8.7 Hz, 1H, H-5(4) Ar), 7.42 (d, J = 8.7 Hz, 2H, H-3 + H-6 Ar), 8.67 (s, 1H, H-9 quinoline); 13C NMR (DMSO-d6, 125 MHz) δ ppm: 25.8, 61.9, 64.0, 111.1, 114.6, 115.8, 118.4, 124.7, 125.9, 129.81, 130.5, 132.7, 134.1, 140.2, 142.7, 157.5, 159.2, 165.5, 177.7, 210.9; HPLC-HRMS (ESI) calc for C21H16FNO3S3 + H+, 446.0350; found, 446.0353 (see Supplementary Materials, Figures S20–S22).

General procedure for synthesis of substituted 1,2-dithiolo[3,4-c]pyrrolo[3,2-i]quinoline-4,5-diones 2n,q: to a solution of the starting compounds 2h (or 2p) (5 mmol) in dry toluene (20 mL), an oxalyl chloride (0.7g, 0.47 mL, 5.5 mmol) was added. The reaction mixture was refluxed for 1.5–2 h while the reaction progress was monitored by TLC. Toluene was distilled off under reduced pressure, the precipitate was filtered, washed with ethanol, and the resulting target products 2n (or 2q) did not require recrystallization.

8,8-Dimethyl-11-thioxo-2,3,8,11-tetrahydro-[1,4]dioxino[2,3-g][1,2]dithiolo[3,4-c]pyrrolo[3,2-i]quinoline-5,6-dione (2n). Dark brown powder (yield 1.58 g, 84%), m.p. > 260 °C; 1H NMR (DMSO-d6, 500 MHz) δ ppm: 2.05 (s, 6H, (CH3)2), 4.28 (q, J = 3.5 Hz, 2H, CH2O), 4.43 (q, J = 3.5 Hz, 2H, CH3O), 9.35 (s, 1H, H-9 quinoline); HPLC-HRMS (ESI) calc for C16H11N2O5S3 + H+, 377.9924; found, 377.9925 (see Supplementary Materials, Figures S23–S24).

10-[1-(3-Methoxyphenyl)iminoo]-7,7-dimethyl-7,10-dihydro-[1,2]dithiolo[3,4-c]pyrrolo[3,2-i]quinoline-4,5-dione (2q). Brown-red powder (yield 1.70 g, 87%), m.p. = 195–196 °C; 1H NMR
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3.2. Virtual Screening

PASS Online allows registered users to receive a prediction of the spectrum of biological activity based on the structural formula of a chemical compound via the Internet (http://www.way2drug.com/passonline, accessed on 1 January 2022) [36]. The forecast results are presented in Table 1 as a list of names of probable activities for the indicated compounds with calculated estimates of the probabilities of presence (Pa) or absence (Pi) of activity, which have values from 0 to 1. Since the probabilities are calculated independently on subsamples of active or inactive compounds, respectively, their sum is not equal to one. The values of Pa and Pi are interpreted as estimates of the measure of whether a substance belongs to the classes of active and inactive compounds, respectively. The greater the value of Pa for a particular activity and the smaller Pi, the higher the chance of detecting this activity in the experiment. If Pa < 0.5, but Pa > Pi, the probability of detecting this type of activity is low, but if the activity is found, then there is a more than 50% chance that the Pa and Pi structure is original [36].

3.3. Biological Evaluation

Study of inhibitory activity. The kinase activity was determined by enzyme-linked immunosorbent assay (ELISA) in polystyrene plates (Costar, 3363) in reaction buffer (20 mM HEPES, pH 7.5, 15 mM MgCl2, 2 mM DTT, 0.2 mM Na3VO4, 0.005% Triton X-100) for 60 min at 30 °C with vigorous stirring. The final concentration of the reaction components: 0.05 µg mL⁻¹ of the corresponding kinase, 5 nM Histon H3 biotinylated substrate (1–21) (Anaspec, 61702), 150 µM ATP (Sigma, A6419), 10 µM test compound, 5% DMSO. The enzymatic reaction was stopped with a buffer containing 20 mM HEPES (Sigma, H4034), pH 7.5, and 150 mM EDTA (Sigma, E5513). Further, to detect the phosphorylated substrate, the reaction mixture was transferred to pre-prepared plates (Nunc, 468667) coated with neutravidin (1 ng per well; Pierce, 31000) and treated with bovine serum albumin (BSA) to block non-specific binding sites. Incubation was carried out for 1 h at room temperature. After washing the plates three times with phosphate buffer saline (PBS, pH 7.4) with Tween-20, they were incubated sequentially with anti-phospho-Histon H3 antibodies (0.3 ng µL⁻¹; Millipore, 04-746) and with specific antibodies conjugated with an enzyme-label (peroxidase) Anti-rabbit IgG, HRP-linked Antibody (titer 1/5000; Cell Signaling, 7074). After completion of each incubation step (60 min at room temperature (~20 °C) and continuous stirring), the plates were washed three times from unbound antibody molecules with a PBS solution with Tween-20 and 100 µL of substrate (3,3′,5,5′-tetramethylbenzidine (TMB) dihydrochloride, Sigma, T8768) prepared according to the manufacturer’s instructions. Before measuring the optical density, the reaction was stopped using 0.5 M H2SO4. The optical density of the solution was determined at λ = 450 nm using a tablet spectrophotometer (TECAN Safire). The data obtained were processed and imported into the HTS-Calc program.

For compounds 2a–c,q, the IC50 value (µmol L⁻¹) was calculated from ten concentration points (in triplicate dilution) obtained twice. The data were analyzed using GraphPad Prism software (v. 3.1).

4. Conclusions

Thus, compounds with pleiotropic activity, including chemoprotective and antitumor activity, were found among previously synthesized and obtained new hybrid and chimeric derivatives of 1,2-dithiolo[3,4-c]quinoline-1-thione using the PASS Online
Experimental testing of the activity towards a number of protein kinases, NPM1-ALK, ALK, EGFR[1858R][790], cRAF[Y340D][Y341D], JAK2, and JAK3, confirmed the calculated predictions. The most active non-selective kinase inhibitors were 8-phenylpirazinylcarboxothioyl and 8-morpholinylcarboxothioyl derivatives of dithioloquinolineimine. In addition, high activity towards protein kinases cRAF[Y340D][Y341D] and JAK3 for the substituted imino-derivative of 1,2-dithiole[3,4-c]pyrrolo[3,2,1-ij]quinoline, which according to in silico predictions was inactive, was determined. This finding indicates the novelty of the structure of the molecule in relation to known drugs.

Considering the need to develop new non-selective kinase inhibitors, derivatives of dithioloquinolinedithione and dithiolequinolineimine require further study as promising preferable.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/molecules27134033/s1. The following supporting information on the data of the HPLC-MS-ESI analysis of compounds 2a–d, and the NMR spectra and data of the HPLC-MS-ESI analysis of compounds 2h, 2i–n, and 2q, can be downloaded online. Figure S1: HPLC–HRMS–ESI spectra of 4,4,5-trimethyl-8-(morpholin-4-ylcarboxothioyl)-4,5-dihydro-1H-[1,2]dithiole[3,4-c]quinoline-1-thione 2a, Figure S2: HPLC–HRMS–ESI spectra of 4,4,5-trimethyl-8-[4-phenylpirazarin-1-yl]carboxothioyl]-4,5-dihydro-1H-[1,2]dithiole[3,4-c]quinoline-1-thione 2b, Figure S3: HPLC–HRMS–ESI spectra of 5-benzyl-4,4-dimethyl-8-(morpholin-4-ylcarboxothioyl)-4,5-dihydro-1H-[1,2]dithiole[3,4-c]quinoline-1-thione 2c, Figure S4: HPLC–HRMS–ESI spectra of 5-benzyl-4,4-dimethyl-8-(piperidin-1-ylcarboxothioyl)-4,5-dihydro-1H-[1,2]dithiole[3,4-c]quinoline-1-thione 2d, Figure S5: NMR 1H spectra of 4,4-dimethyl-4,5,8,9-tetrahydro-1H-[1,4]dioxino[2,3-g][1,2]dithiole[3,4-c]quinoline-1-thione 2e, Figure S6: NMR 13C spectra of 4,4,5,8,9-tetrahydro-1H-[1,4]dioxino[2,3-g]-[1,2]dithiole[3,4-c]quinoline-1-thione 2f, Figure S7: HPLC–HRMS–ESI spectra of 4,4-dimethyl-4,5,8,9-tetrahydro-1H-[1,4]dioxino[2,3-g]-[1,2]dithiole[3,4-c]quinoline-1-thione 2h, Figure S8: NMR 1H spectra of 8-methoxy-4,4-dimethyl-5-(2-thienylcarbonyl)-4,5-dihydro-1H-[1,2]dithiole[3,4-c]quinoline-1-thione 2i, Figure S9: NMR 13C spectra of 8-methoxy-4,4-dimethyl-5-(2-thienylcarbonyl)-4,5-dihydro-1H-[1,2]dithiole[3,4-c]quinoline-1-thione 2j, Figure S10: HPLC–HRMS–ESI spectra of 8-methoxy-4,4-dimethyl-5-(2-thienylcarbonyl)-4,5-dihydro-1H-[1,2]dithiole[3,4-c]quinoline-1-thione 2k, Figure S11: NMR 1H spectra of 1-[2-(8-methoxy-4,4-dimethyl-1-thioxo-1,4-dihydro-5H-[1,2]dithiole[3,4-c]quinolin-5-yl)-2-oxoethyl]pyrrolidine-2,5-dione 2l, Figure S12: NMR 13C spectra of 1-[2-(8-methoxy-4,4-dimethyl-1-thioxo-1,4-dihydro-5H-[1,2]dithiole[3,4-c]quinolin-5-yl)-2-oxoethyl]pyrrolidine-2,5-dione 2m, Figure S13: HPLC–HRMS–ESI spectra of 1-[2-(8-methoxy-4,4-dimethyl-1-thioxo-1,4-dihydro-5H-[1,2]dithiole[3,4-c]quinolin-5-yl)-2-oxoethyl]pyrrolidine-2,5-dione 2n, Figure S14: NMR 1H spectra of 2-[2-(8-methoxy-4,4-dimethyl-1-thioxo-1,4-dihydro-5H-[1,2]dithiole[3,4-c]quinolin-5-yl)-2-oxoethyl]hexahydro-1H-isindole-1,3(2H)-dione 2o, Figure S15: NMR 13C spectra of 2-[2-(8-methoxy-4,4-dimethyl-1-thioxo-1,4-dihydro-5H-[1,2]dithiole[3,4-c]quinolin-5-yl)-2-oxoethyl]hexahydro-1H-isindole-1,3(2H)-dione 2p, Figure S16: HPLC–HRMS–ESI spectra of 2-[2-(8-methoxy-4,4-dimethyl-1-thioxo-1,4-dihydro-5H-[1,2]dithiole[3,4-c]quinolin-5-yl)-2-oxoethyl]hexahydro-1H-isindole-1,3(2H)-dione 2q, Figure S17: NMR 1H spectra of 5-[3-chloro-1-benzothien-2-yl]carbonyl]-7-methoxy-4,4-dimethyl-4,5-dihydro-1H-[1,2]dithiole[3,4-c]quinoline-1-thione 2r, Figure S18: NMR 13C spectra of 5-[3-chloro-1-benzothien-2-yl]carbonyl]-7-methoxy-4,4-dimethyl-4,5-dihydro-1H-[1,2]dithiole[3,4-c]quinoline-1-thione 2s, Figure S19: HPLC–HRMS–ESI spectra of 5-[3-chloro-1-benzothien-2-yl]carbonyl]-7-methoxy-4,4-dimethyl-4,5-dihydro-1H-[1,2]dithiole[3,4-c]quinoline-1-thione 2t, Figure S20: NMR 1H spectra of 5-(2-fluorobenzoyl)-4,4-dimethyl-4,5,8,9-tetrahydro-1H-[1,4]dioxino[2,3-g]-[1,2]dithiole[3,4-c]quinoline-1-thione 2u, Figure S21: NMR 13C spectra of 5-(2-fluorobenzoyl)-4,4-dimethyl-4,5,8,9-tetrahydro-1H-[1,4]dioxino[2,3-g]-[1,2]dithiole[3,4-c]quinoline-1-thione 2v, Figure S22: HPLC–HRMS–ESI spectra of 5-(2-fluorobenzoyl)-4,4-dimethyl-4,5,8,9-tetrahydro-1H-[1,4]dioxino[2,3-g]-[1,2]dithiole[3,4-c]quinoline-1-thione 2w, Figure S23: NMR 1H spectra of 8,8-dimethyl-11-thioxo-2,3,8,11-tetrahydro[1,4]dioxino[2,3-g]-[1,2]dithiole[3,4-c]pyrrolo[3,2,1-ij]quinoline-5,6-dione 2x, Figure S24: HPLC–HRMS–ESI spectra of 8,8-dimethyl-11-thioxo-2,3,8,11-tetrahydro[1,4]dioxino[2,3-g]-[1,2]dithiole[3,4-c]pyrrolo[3,2,1-ij]quinoline-5,6-dione 2y, Figure S25: NMR 1H spectra of 10-[3-methoxyphenyl]limino]-7,7-dimethyl-7,10-dihydro[1,2]dithiole[3,4-c]pyrrolo[3,2,1-ij]quinoline-4,5-dione 2z, Figure S26: NMR 13C spectra of 10-[3-methoxyphenyl]limino]-7,7-dimethyl-7,10-dihydro[1,2]dithiole[3,4-c]pyrrolo[3,2,1-ij]quinoline-4,5-dione 2a.
iolo[3,4-c]pyrrolo[3,2-ij]quinoline-4,5-dione 2q. Figure S27: HPLC–HRMS–ESI spectra of 10-[(3-methoxyphenyl)limino]-7,7-dimethyl-7,10-dihydro[1,2]dithiolo[3,4-c]pyrrolo[3,2-1-ij]quinoline-4,5-dione 2q.

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