Synthesis and Dual Histamine H₁ and H₂ Receptor Antagonist Activity of Cyanoguanidine Derivatives

Bassem Sadek 1,*, Rudi Alisch 2, Armin Buschauer 3 and Sigurd Elz 3

1 Department of Pharmacology and Therapeutics, College of Medicine and Health sciences, UAE University, Al-Ain, P.O. Box 17666, UAE
2 Institute of Pharmacy, Free University of Berlin, Königin-Luise-Str. 2-4, Berlin D-14195, Germany
3 Institute of Pharmacy, University of Regensburg, University Str. 31, Regensburg D-93053, Germany

* Author to whom correspondence should be addressed; E-Mail: bassem.sadek@uaeu.ac.ae; Tel.: +971-371-371-512; Fax: +971-371-672-033.

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Abstract: Premedication with a combination of histamine H₁ receptor (H₁R) and H₂ receptor (H₂R) antagonists has been suggested as a prophylactic principle, for instance, in anaesthesia and surgery. Aiming at pharmacological hybrids combining H₁R and H₂R antagonistic activity, a series of cyanoguanidines was synthesized by linking mepyramine-type H₁R antagonist substructures with roxatidine-, tiotidine-, or ranitidine-type H₂R antagonist moieties. N-desmethylmepyramine was connected via a poly-methylene spacer to a cyanoguanidine group as the “urea equivalent” of the H₂R antagonist moiety. The title compounds were screened for histamine antagonistic activity at the isolated ileum (H₁R) and the isolated spontaneously beating right atrium (H₂R) of the guinea pig. The results indicate that, depending on the nature of the H₂R antagonist partial structure, the highest H₁R antagonist potency resided in roxatidine-type compounds with six methylene groups in length (compound 21), and tiotidine-type compounds irrespective of the alkyl chain length (compounds 28, 32, 33). N-cyano-N'-[2-[[2-guanidino-4-thiazolyl]methyl]thio]ethyl]-N''-[2-[N-[2-[N-(4-methoxybenzyl)-N-(pyridyl)-amino]ethyl]-N-methylamino]ethyl] guanidine (25, pKᵦ values: 8.05 (H₁R, ileum) and 7.73 (H₂R, atrium) and the homologue with the mepyramine moiety connected by a six-membered chain to the tiotidine-like partial structure (compound 32, pKᵦ values: 8.61 (H₁R) and 6.61 (H₂R) were among the most potent hybrid compounds. With respect to the development of a potential pharmacotherapeutic agent, structural optimization seems possible through selection of other H₁R and H₂R pharmacophoric moieties with mutually affinity-enhancing properties.
Keywords: dual H₁/H₂ receptor antagonists; mepyramine; roxatidine; tiotidine; ranitidine

1. Introduction

The biogenic amine histamine mediates its effects via four histamine receptor subtypes, termed H₁, H₂, H₃, and H₄ receptors (HₓR) [1–3]. The histamine receptors belong to class A of the superfamily of G-protein-coupled receptors (GPCRs). As an autacoid and neurotransmitter, histamine is involved in numerous physiological and pathophysiological processes. Antagonists of the H₃R [4] and the most recently discovered H₄R [5] are still under investigation as potential drugs, e.g., for the treatment of CNS disorders and inflammatory diseases, respectively [6]. By contrast, H₁R and H₂R antagonists are well established therapeutic agents for decades. Numerous pathophysiological responses to histamine released from mast cells and basophils through immunological or non-immunological mechanisms are mediated by the H₁R, for instance, vasodilatation via nitric oxide release, increase in capillary permeability, contraction of smooth muscles, e.g., in gut and bronchi. The first H₁R blockers [7] have been described as “antihistamines” more than 70 years ago, and especially the newer non-sedating H₁R antagonists are widely used in the treatment of allergic conditions [7]. Stimulation of gastric acid secretion is the most prominent physiological effect of H₂R stimulation. In the 1970s the development of the H₂R antagonists revolutionized the treatment of gastric and duodenal ulcers [8]. Histamine can also induce cardiovascular effects via H₂Rs, e.g., increase in heart rate and cardiac contractility as well as vasodilatation [9].

The combined administration of H₁R and H₂R antagonists has been suggested, for example, as a prophylactic principle in anaesthesia, for the prevention of hypersensitivity reactions to drugs, e.g., in cancer chemotherapy, or for the treatment of skin diseases [10–15]. Histamine release during anaesthesia and surgery is still a widely known problem in clinical practice [10]. The ratio of anaphylactic/anaphylactoid reaction incidents is about 20%–30%, and life threatening reactions are observed in 0.1%–0.5% of all cases [16]. Cardiac arrhythmias induced by activation of H₁ and H₂ receptors are among the most serious consequences of mast cell degranulation. Several studies have shown the effectiveness of a premedication with a combination of H₁R and H₂R antagonists, for example dimetindene plus cimetidine [10,17–20].

The commercially available H₁ and H₂ antihistamines differ considerably concerning physicochemical and pharmacokinetic properties. A combination of both qualities of action in one hybrid drug could be of therapeutic advantage, for instance, in terms of pharmacokinetics. Such hybrid antihistamines could also be of potential interest in other indications, e.g., in the therapeutic management of atopic dermatitis. Icotidine (SK&F 93319), derived from the isocytosine series of H₂R antagonists, was the first substance reported to possess nearly equipotent antagonist activity at both H₁R and H₂R [21] (Figure 1).
Previously, urea-type H$_1$/H$_2$ receptor antagonists such as compound 1 were synthesized and pharmacologically investigated [22–24]. Lessons learned, in particular from the H$_4$R and H$_3$R, suggest that differences between H$_x$R species orthologues should be taken into account [2,3]. Nevertheless, isolated guinea-pig organs have been used as standard pharmacological models to characterize H$_1$R and H$_2$R antagonists for many decades, and the data gained from these investigations provided a reliable basis for numerous successfully marketed histamine receptor antagonists. Therefore, with respect to potential studies in translational animal models, the synthesized compounds were investigated on the guinea pig isolated ileum and the spontaneously beating right atrium. The urea derivative 1, containing a three-membered carbon chain as a spacer and fluoropheniramine substructure, had a pK$_B$ value of 8.21 at the isolated guinea pig ileum and proved to be more active than pheniramine at the H$_1$R. However, H$_2$R antagonist activity (guinea pig atrium: pK$_B$ 6.68) was found to be moderate, presumably due to introduction of a basic centre close to the polar group [23] (Figure 1).

As mepyramine (pA$_2$ 9.07 ± 0.03) [25] is more potent than pheniramine-like H$_1$R antagonists, in the present study, the mepyramine partial structure was used as a building block for the synthesis of hybrid compounds. The H$_1$R antagonist pharmacophoric moiety was connected via a carbon chain (spacer) of variable length and a cyanoguanidine group with the H$_2$R antagonist partial structure of ranitidine, tiotidine, and cimetidine (Figure 2). Thereby, the cyanoguanidine moiety, a characteristic “urea equivalent” [26,27] of H$_2$R antagonists such as cimetidine, was kept unchanged as a bioisosteric replacement of the polar moieties present in ranitidine, tiotidine and ranitidine, respectively.

Figure 2. General structure of spacer-linked combined H$_1$/H$_2$ receptor antagonists.
2. Results and Discussion

2.1. Chemistry

The cyanoguanidines 14–35 were synthesized starting from triamines 4–13 which can be easily prepared from the secondary amines 3a and 3b through alkylation with acrylonitrile or ω-haloalkanenitriles of different chain lengths followed by reduction with LiAlH₄ in diethyl ether (Scheme 1). Secondary amines 3a and 3b were obtained by acylation of primary amines 2a and 2b with ethyl chloroformate and subsequent reduction with LiAlH₄ in tetrahydrofuran [23,24].

Scheme 1. Synthetic pathway for intermediates 4–13.

![Synthetic pathway for intermediates 4–13.](image)

| No. | R¹ | y |
|-----|----|---|
| 4   | OCH₃| 2 |
| 5   | F   | 2 |
| 6   | OCH₃| 3 |
| 7   | OCH₃| 3 |
| 8   | F   | 4 |
| 9   | OCH₃| 4 |
| 10  | F   | 5 |
| 11  | OCH₃| 6 |
| 12  | F   | 6 |
| 13  | F   | 7 |

Reagents and Conditions: 

| Reagent | Condition |
|---------|-----------|
| a       | Ethylenediamine, pyridine, reflux, 3 h; NaH, 4-methoxybenzylchloride (for 2a), 4-fluorobenzylchloride (for 2b), DMSO, room temperature, 12 h; NaOH, ethyl chloroformate, THF, room temperature; (i) Acrylonitrile or corresponding ω-chloroalkylnitrile, KI, excess Na₂CO₃, 60 °C, 2 h; (ii) Lithium aluminum hydride (50% excess), diethyl ether, room temperature, 2 h. |

The triamines 4–13 were allowed to react with the isourea intermediates A1–D1 (Scheme 2) to yield the cyanoguanidines 14–35 [22–24,28–30]. Addition of the roxatidine-like primary amines A and B resulted in 14–23 and 24, respectively, while addition of C and D afforded the compounds 25–34 and 35, respectively [24].

2.2. Pharmacology

The synthesized hybrid compounds 14–35 were investigated for histamine H₁R and H₂R antagonism at the isolated guinea pig ileum (H₁R) and the isolated spontaneously beating guinea pig right atrium (H₂R). The results are summarized in Table 1.
Compounds 14–35 showed histamine H1R antagonistic activities on guinea pig ileum with pK_B values in the range from 6.8 (compound 14) to 8.6 (compounds 28, 32). Although the activity increased with the length of the polymethylene spacer in case of the methoxy-substituted derivatives bearing a roxatidine-like H2-antagonist moiety (compound 14 vs. 16, 18 and 21), a clear general tendency was not obvious, neither for the corresponding fluorinated analogues nor for the guanidinothiazoles 25–34. Highest H1R antagonistic activity resided in the latter group of compounds. Though an affinity-increasing effect was not evident, it should be noted that the interactions with the H1R were not dramatically affected in an adverse manner by the H2R antagonist moiety. The most potent H1R antagonists achieved K_B values in the one-digit nanomolar range corresponding to about 30% of the activity of mepyramine at the guinea-pig ileum.
Table 1. Histamine H₁ and H₂ receptor antagonism of cyanoguanidines 14–35.

| Compound     | R¹ | y | R² | pK_B a | rel. activity | conc. [M] | pK_B a | rel. activity | conc. [M] |
|--------------|----|---|----|--------|---------------|-----------|--------|---------------|-----------|
| Mepyramine   | -  | - | -  | 9.07 b | 100           | -         | -      | -             | -         |
| Cimetidine   | -  | - | -  | 7.77  | 5.0           | 7.49 1230 | -      | -             | -         |
| Icotidine c  | -  | - | -  | 8.21  | 14 0.3 × 10⁻⁶ | 6.89 190 10⁻⁶–0.3 × 10⁻⁵ | -      | -             | -         |
| 14 OCH₃      | 2  | A | -  | 6.88  | 1 0.3 × 10⁻⁶ | 6.63 170 10⁻⁷–0.3 × 10⁻⁶ | -      | -             | -         |
| 15 F         | 2  | A | -  | 7.83  | 6 10⁻⁷ | 5.97 37 0.3 × 10⁻⁵ | -      | -             | -         |
| 16 OCH₃      | 3  | A | -  | 7.81  | 6 10⁻⁷–0.3 × 10⁻⁶ | 6.37 93 0.3 × 10⁻⁶ | 7.49 1230 | -      | -             | -         |
| 17 F         | 3  | A | -  | 7.77 e | 5 10⁻⁷–0.3 × 10⁻⁶ | 6.0 40 0.3 × 10⁻⁶–0.3 × 10⁻⁶ | -      | -             | -         |
| 18 OCH₃      | 4  | A | -  | 7.76  | 5 10⁻⁸–0.3 × 10⁻⁷ | 6.38 96 0.3 × 10⁻⁷ | 7.49 1230 | -      | -             | -         |
| 19 F         | 4  | A | -  | 7.75  | 5 10⁻⁷–0.3 × 10⁻⁷ | 5.76 e 23 10⁻⁵–0.3 × 10⁻⁵ | -      | -             | -         |
| 20 F         | 5  | A | -  | 7.95  | 7 10⁻⁷ | 6.68 190 10⁻⁶–0.3 × 10⁻⁵ | -      | -             | -         |
| 21 OCH₃      | 6  | A | -  | 8.42  | 22 10⁻⁷–0.3 × 10⁻⁷ | 6.43 107 0.3 × 10⁻⁷ | 7.49 1230 | -      | -             | -         |
| 22 F         | 6  | A | -  | 8.06  | 10 10⁻⁷–0.3 × 10⁻⁷ | 6.41 102 10⁻⁶ | -      | -             | -         |
| 23 F         | 7  | A | -  | 7.93  | 7 10⁻⁷–0.3 × 10⁻⁷ | 6.17 59 10⁻⁶–0.3 × 10⁻⁷ | -      | -             | -         |
| 24 F         | 4  | B | -  | 7.65  | 4 10⁻⁷–0.3 × 10⁻⁷ | 6.07 47 10⁻⁶ | -      | -             | -         |
| 25 OCH₃      | 2  | C | -  | 8.05  | 10 10⁻⁷–0.3 × 10⁻⁷ | 7.37 933 10⁻⁷ | 7.49 1230 | -      | -             | -         |
| 26 F         | 2  | C | -  | 7.94  | 7 10⁻⁸–0.3 × 10⁻⁷ | 6.62 166 10⁻⁷ | 7.49 1230 | -      | -             | -         |
| 27 OCH₃      | 3  | C | -  | 8.21  | 14 10⁻⁸–0.3 × 10⁻⁷ | 6.52 132 10⁻⁷ | -      | -             | -         |
| 28 F         | 3  | C | -  | 8.62  | 36 10⁻⁸–0.3 × 10⁻⁸ | 6.47 118 0.3 × 10⁻⁶–0.3 × 10⁻⁵ | -      | -             | -         |
| 29 OCH₃      | 4  | C | -  | 7.91  | 7 0.3 × 10⁻⁷ | 7.00 398 10⁻⁷–0.3 × 10⁻⁶ | -      | -             | -         |
| 30 F         | 4  | C | -  | 8.32  | 18 10⁻⁸–0.3 × 10⁻⁸ | 6.28 76 10⁻⁶ | 7.49 1230 | -      | -             | -         |
| 31 F         | 5  | C | -  | 8.36  | 20 10⁻⁸–0.3 × 10⁻⁷ | 6.51 129 10⁻⁶–0.3 × 10⁻⁵ | -      | -             | -         |
| 32 OCH₃      | 6  | C | -  | 8.61  | 35 10⁻⁷–0.3 × 10⁻⁷ | 6.61 162 10⁻⁷–0.3 × 10⁻⁶ | -      | -             | -         |
| 33 F         | 6  | C | -  | 8.44  | 23 0.3 × 10⁻⁷ | 6.52 132 10⁻⁶ | 7.49 1230 | -      | -             | -         |
| 34 F         | 7  | C | -  | 8.15  | 12 10⁻⁸ | 7.02 417 10⁻⁶–0.3 × 10⁻⁶ | -      | -             | -         |
| 35 OCH₃      | 3  | D | -  | 7.95  | 7 10⁻⁸–0.3 × 10⁻⁷ | 5.22 e 7 10⁻⁵ | -      | -             | -         |

* Determined at the isolated ileum (H₁) and right atrium (H₂) of the guinea pig; pK_B mean values, S.E.M. for ileum within ±0.2 by 16, 17, 18, and 19; ±0.1 by all other compounds; pK_B mean values, S.E.M. for atrium within ±0.2 by 14, and 17; ±0.1 by all other compounds; rel. activity, % activity relative to mepyramine, icotidine or cimetidine, respectively; antag. conc., antagonist concentrations used; b [25]; c [21]; d [23]; e At 0.3 × 10⁻⁶ M, depression of the concentration response curve by 15%, 30%, 15%, and 20% for 15, 17, and 19, respectively.

It is well known from numerous investigational and commercially available H₂R antagonists (e.g., cimetidine, ranitidine or tiotidine) that, comparing the numeric values, the highest antagonistic activities achievable at the guinea pig right atrium (Table 1) are usually by one to two orders of
magnitude lower than at the guinea pig ileum. The same holds for the investigated hybrid compounds. Highest H2R antagonistic activity resided in guanidinothiazole 25 with a pK_B value of 7.37, corresponding to nine-fold higher activity than cimetidine or three-fold lower activity than tiotidine, respectively. Among the cyanoguanidines substituted with roxatidine-like partial structures (compounds 14–23), lengthening of the spacer from ethylene to pentamethylene decreased both antagonist activities on H1 and H2 receptors. However, cyanoguanidine derivative 21 (pK_B ileum, 8.42, pK_B atrium, 6.43) with a hexamethylene spacer separating the basic center from the polar cyanoguanidine moiety was approximately two times more active on H1 receptors, and the H2 antagonistic activity was in the same range as that of reference compounds 1 or cimetidine. Moreover, the presence of a methoxy group at p-position of the mepyramine partial structure seemed to be of advantage over fluorine substitution. Compared to 22, the H1R antagonist activity of 21 was approximately twofold higher whereas the H2R antagonist remained unchanged. Further extension of the spacer up to seven methylene groups in the H1R antagonist partial structure or up to four methylene groups in the H2R antagonist moiety decreased both H1R and H2R antagonistic activities as demonstrated by cyanoguanidine derivatives 23 (pK_B ileum, 7.93, pK_B atrium, 6.17) and 24 (pK_B ileum, 7.65, pK_B atrium, 6.07), respectively. Thus, compound 21 represents the optimum in terms of combined H1R/H2R antagonistic activity among the piperidinomethylphenoxyalkylcyanoguanidines.

A further increase in potency was achieved by replacement of the roxatidine-like moiety with a guanidinothiazole (compounds 25–34) as in tiotidine, resulting in the highest histamine H1R and H2R antagonistic activities among this series of pharmacological hybrids. Compound 32 with a hexamethylene spacer was found to be up to two times more potent at both H1R and H2R compared to the roxatidine-like analog 21. In addition, the results revealed that a p-methoxy moiety (compound 32), unlike p-fluoro substituent (compound 33), potentiates the antagonistic activities at both H1 and H2 receptors. Remarkably, further extension of the spacer up to 7 methylene groups resulted in cyanoguanidine derivative 34, the most potent H2R antagonist amid the title compounds. However, H1R antagonistic decreased up to 5-fold compared to compounds 32 and 33. The cyanoguanidine derivative 35, bearing a ranitidine-like moiety, did not show an activity-enhancing effect compared to the corresponding parent compounds. Compound 25 with an ethylene spacer and tiotidine partial structure revealed the highest H2R antagonistic activity among the presented series of compounds.

In summary, the combination of the mepyramine substructure with a tiotidine-like guanidinothiazole turned out to be most suitable to obtain pharmacological hybrid with activities comparable to those of commercially available selective H1R and H2R antagonists.

3. Experimental

3.1. Chemistry

3.1.1. General Conditions

Melting points are uncorrected and determined in open capillaries in a Buechi 512 Dr. Tottoli apparatus. 1H-NMR spectra were recorded on a Bruker WC 300 spectrometer with tetramethylsilane (TMS) as internal standard. Chemical shifts are reported in ppm downfield from internal tetramethylsilane as reference. 1H-NMR signals are reported in order: multiplicity (s, single;
d, doublet; t, triplet; q, quintet; m, multiplet; br, broad; *, exchangeable by D₂O), number of protons, and approximate coupling constants in Hertz. Elemental analyses were performed on Perkin-Elmer 240B and 240C instruments. Analyses (C, H, N) indicated by the symbols of elements were within ±0.4% of the theoretical values. Chromatographic separations were done by rotation planar chromatography (centrifugal layer chromatography) using a Chromatotron Model 7924 (Harrison Research, Muttenz, Switzerland) with 4-mm layers of silica gel 60 PF₂₅₄ containing gypsum (Merck). To avoid tailing, a saturated ammonia atmosphere was produced by passing through a stream of anhydrous gaseous ammonia via the “inert gas inlet” of the Chromatotron. EI-mass spectra were recorded using Finnigan MAT CH7A (70 eV), Finnigan MAT 711 (80 eV), or Kratos MS 25 RF (70 eV). FAB-MS spectra were recorded on Finnigan MAT CH5DF instrument (xenon, DMSO)/glycerol). The following abbreviations are used: DMF, N,N-dimethylformamide; EtOH, ethanol; Et₂O, diethyl ether; MeOH, methanol; Me₂SO, dimethyl sulfoxide; Ph, phenyl; * exchangeable with D₂O; C₂H₂O₄, oxalic acid; Py, pyridyl; Pip, piperidyl; TA, C₄H₆O₆, tartaric acid; C₂₀H₁₈O₈, (−)-di-p-tolyltartaric acid; decomp, decomposition.

3.1.2. Synthesis of Primary Amines 2a and 2b

2-Bromopyridine (109.30 mmol) and ethylenediamine (546 mmol) were refluxed in pyridine for 3 h. Excess of ethylenediamine was evaporated under reduced pressure, water was added, the pH value was adjusted to >11 using NaOH (1N), and the 2-(2-aminoethylamino)-pyridine was extracted with chloroform [29]. After evaporation of chloroform, the product was purified by means of a Chromatotron (chloroform/methanol, 97/3, V/V, ammonia atmosphere), and used in the following reaction without further purifications. The primary amines 2a and 2b were synthesized by suspending 2-(2-aminoethylamino)pyridine (73.40 mmol) with sodium hydride (60% oil suspension, 64.50 mmol) in DMSO under nitrogen atmosphere. The reaction temperature was increased gradually and kept at 85 °C until hydrogen evolution ceased. For synthesis of 2a and 2b, 4-methoxybenzylchloride and 4-fluorobenzylchloride, respectively, were added, and the mixture was stirred for 12 h at room temperature. Subsequently, water (500 mL) was added, 2a and 2b were extracted with diethyl ether at pH > 11, combined extracts were dried over anhydrous potassium carbonate, and the solvent was evaporated under reduced pressure yielding the oily products 2a and 2b, which were used in the following reaction to prepare 3a and 3b without further purification.

3.1.3. Synthesis of Secondary Amines 3a and 3b

For synthesis of 3a and 3b, 43.64 mmol of the corresponding primary amine 2a and 2b were dissolved in diethyl ether, NaOH (10%) was added and the solution was kept over ice-bath. Ethyl chloroformate was added gradually to the two-phase system. After complete reaction, the diethyl ether layer was separated, dried over sodium sulfate, and evaporated under reduced pressure to yield the oily products 3a and 3b, respectively [22–24,28].
3.1.4. General Procedure for the Synthesis of the Diamines 4–13

As previously described, a mixture of the corresponding secondary amine 3a or 3b (10–30 mmol), an equimolar amount of the pertinent \( \omega \)-halonitrile, a catalytic amount of potassium iodide and a 100% excess of sodium carbonate was stirred for 2 h at 60 °C in 20 mL of a mixture of acetonitrile and dimethylformamide (1:1 (V/V) in case of chloroacetonitrile, 9:1 (V/V) in case of homologous halonitriles) [22–24,28]. Subsequently, water was added, the mixture was extracted with toluene, the combined extracts were dried over sodium sulfate and evaporated under reduced pressure yielding the corresponding oily aminonitriles as intermediates, which were dissolved in 20 mL of anhydrous diethyl ether and dropped to an ice-cold suspension of lithium aluminium hydride (50% excess). After 2 h stirring at room temperature the reaction mixture was hydrolyzed by addition of water followed by 3 mL of a 10% aqueous sodium hydroxide solution. The resulting diamines 4–13 were purified chromatographically (Chromatotron, eluent: chloroform/methanol, gradient from 99:1 to 90:10 (V/V), ammonia atmosphere), and were used in the following reactions for synthesis of cyanoguanidines 14–35 [22–24,28].

3.1.5. General Procedure for the Synthesis of Cyanoguanidines 14–35

The pertinent \( N \)-cyano \( O \)-phenyl isoureas (2–4 mmol) A1–D1, which were prepared by stirring of equivalent amounts of A–D with diphenyl \( N \)-cyanocarbonimidate for 2 h, and equimolar amounts of the respective diamines 4–13 in 30 mL of anhydrous acetonitrile, were heated under reflux for 16 h. The mixture was evaporated to dryness and the cyanoguanidines 14–35 were isolated chromatographically (Chromatotron, chloroform: methanol, gradient from 99:1 to 90:10, ammonia atmosphere) [22–24,28].

\( N \)-Cyano-\( N' \)-[2-[\( N \)-[2-[\( N \)-(4-methoxybenzyl)-\( N \)-(2-pyridyl)amino]ethyl]-\( N \)-methylamino]ethyl]–\( N'' \)-[3-[3-(piperidinomethyl)phenoxy]propyl]guanidine (14). Yield: 65%, mp: 88–89 °C; \( ^{1} \)H-NMR (CDCl3): \( \delta \) ppm = 1.41–1.42 (m, 2H, PipH); 1.53–1.57 (m, 4H, PipH), 1.98 (m, 2H, CH2); 2.25 (s, 3H, N-CH3); 2.34 (br, 4H, PipH); 2.57–2.62 (m, 4H, 2(CH2-NCH3)); 3.20–3.21 (m, 2H, CH2-NH); 3.30–3.32 (m, 2H, CH2-NH); 3.40 (s, 2H, Ph-CH2-Pip); 3.66 (t, \( J = 7 \), 2H, CH2-N); 3.76 (s, 3H, Ph-OCH3); 3.97 (t, \( J = 6 \), 2H, Ph-O-CH2); 4.60 (s, 2H, Ph-CH2-N); 5.85 (br, 1H*, NH); 6.40–6.43 (d, \( J = 9 \), 1H aromatic, Py5-H); 6.51–6.55 (m, 1H aromatic, Py3-H); 6.73–6.75 (d, \( J = 8.5 \), 1H aromatic, 4-H); 6.81–6.84 (d, \( J = 8 \), 2H aromatic, 2-H); 6.87–6.90 (d, \( J = 8 \), 2H aromatic, 2-H); 7.10–7.13 (m, 2H aromatic, 3-H); 7.10–7.13 (br, 1H*, NH); 7.17 (t, \( J = 8 \), 1H aromatic, 3-H); 7.36 (m, 1H aromatic, Py4-H); 8.12–8.13 (d, \( J = 3 \), 1H aromatic, Py6-H); MS: m/z (%) 612 (M\(^+\), 1), 121 (100); Anal. Calcd. for C\(_{35}\)H\(_{48}\)N\(_{8}\)O\(_{2}\)-H\(_{2}\)O: C, 66.60; H, 7.99; N, 17.80. Found: C, 66.70; H, 7.82; N, 17.45.

\( N \)-Cyano-\( N' \)-[2-[\( N \)-[2-[\( N \)-(4-fluorobenzyl)-\( N \)-(2-pyridyl)amino]ethyl]-\( N \)-methylamino]ethyl]–\( N'' \)-[3-[3-(piperidinomethyl)phenoxy]propyl]guanidine (15). Yield: 33%, mp: 106–108 °C; \( ^{1} \)H-NMR (CDCl3): \( \delta \) ppm = 1.44 (m, 2H, PipH); 1.61 (m, 4H, PipH), 1.90 (m, 2H, CH2); 2.31 (s, 3H, N-CH3); 2.59–2.64 (br, 4H, PipH); 2.71 (m, 4H, 2(CH2-NCH3)); 3.24 (m, 2H, CH2-NH); 3.37–3.47 (m, 2H, CH2-NH); 3.63 (m, 2H, CH2-N); 3.83 (s, 2H, Ph-CH2-Pip); 3.96 (t, 2H, Ph-O-CH2); 4.18 (s, 6H, TA); 4.72 (s, 2H, Ph-CH2-N); 5.20–5.60 (br, 6H*, TA-OH); 6.54 (m, 2H aromatic, Py5-H, Py3-H); 6.86–6.88 (m, 3H aromatic, Py4-H); 7.10–7.13 (m, 2H aromatic, 3-H); 7.10–7.13 (br, 1H*, NH); 7.17 (t, \( J = 8 \), 1H aromatic, 3-H); 7.36 (m, 1H aromatic, Py4-H); 8.12–8.13 (d, \( J = 3 \), 1H aromatic, Py6-H); MS: m/z (%) 612 (M\(^+\), 1), 121 (100); Anal. Calcd. for C\(_{35}\)H\(_{48}\)N\(_{8}\)O\(_{2}\)-H\(_{2}\)O: C, 66.60; H, 7.99; N, 17.80. Found: C, 66.70; H, 7.82; N, 17.45.
aromatic, 4-H, 2-H); 6.86–6.88 (br, 3H*, 3(H-(N+)); 6.92 (m, 2H aromatic, 2-H); 6.92 (br, 1H*, NH); 7.11 (m, 2H aromatic, 3-H); 7.25 (m, 2H aromatic, 3-H); 7.25 (br, 1H*, NH); 7.45 (m, 1H aromatic, Py4-H); 8.06 (d, 1H aromatic, Py6-H); 10.50 (br, 3H*, TA-COOH); MS: m/z (%) 601 (M+, 7), 76 (100); Anal. Calcd. for C34H45FN8O·3C4H6O6: C, 52.60; H, 6.04; N, 10.70. Found: C, 52.50; H, 6.27; N, 10.60.

N-Cyano-N’-[3-[N-[2-[N-(4-methoxybenzyl)-N-(2-pyridyl)amino]ethyl]-N-methylamino]propyl]-N”-[3-[piperidinomethyl]phenoxy]propyl]guanidine (16). Yield: 51%, mp: 62–65 °C; 1H-NMR (CDCl3): δ ppm = 1.41–1.42 (m, 2H, PipH); 1.53–1.57 (m, 4H, PipH); 1.66 (m, 2H, CH2); 2.25 (s, 3H, N-CH3); 2.34 (br, 4H, PipH); 2.57–2.62 (m, 4H, 2(CH2-NCH3)); 3.20–3.21 (m, 2H, CH2-N); 3.30–3.32 (m, 2H, CH2-N); 3.40 (s, 2H, Ph-CH2-Pip); 3.66 (t, J = 7, 2H, CH2-N); 3.76 (s, 3H, Ph-OCH3); 3.97 (t, J = 6, 2H, Ph-O-CH2); 4.60 (s, 2H, Ph-CH2-N); 5.85 (br, 1H*, NH); 6.40–6.43 (d, J = 9, 1H aromatic, Py5-H); 6.51–6.55 (m, 1H aromatic, Py3-H); 6.73–6.75 (d, J = 8.5, 1H aromatic, 4-H); 6.81–6.84 (d, J = 8, 2H aromatic, 2-H); 6.87–6.90 (d, J = 8, 2H aromatic, 2-H); 7.10–7.13 (m, 2H aromatic, 3-H); 7.17 (t, J = 8, 1H aromatic, 3-H); 7.36 (m, 1H aromatic, Py4-H); 8.12–8.13 (d, J = 3, 1H aromatic, Py6-H); MS: m/z (%) 627 (M+, 6), 241 (11), 121 (100); Anal. Calcd. for C36H50N8O2: C, 69.0; H, 8.04; N, 17.90. Found: C, 68.80; H, 8.27; N, 17.70.

N-Cyano-N’-[3-[N-[2-[N-(4-fluorobenzyl)-N-(2-pyridyl)amino]ethyl]-N-methylamino]propyl]-N”-[3-[piperidinomethyl]phenoxy]propyl]guanidine (17). Yield: 57%, mp: 65–68 °C; 1H-NMR (CDCl3): δ ppm = 1.43 (m, 2H, PipH); 1.57–1.61 (m, 4H, PipH); 1.68 (m, 2H, CH2); 1.99–2.05 (m, 2H, CH2); 2.28 (s, 3H, N-CH3); 2.40 (br, 4H, PipH); 2.51 (t, J = 7, 2H, CH2-NCH3); 2.58 (t, J = 7, 2H, CH2-NCH3); 3.25–3.26 (m, 2H, CH2-NH); 3.39 (m, 2H, CH2-NH); 3.45 (s, 2H, Ph-CH2-Pip); 3.70 (t, J = 7.5, 2H, CH2-N); 4.03 (t, J = 6, 2H, Ph-O-CH2); 4.63 (s, 2H, Ph-CH2-N); 6.41 (d, J = 9, 1H aromatic, Py5-H); 6.55 (t, J = 6, 1H aromatic, Py3-H); 6.75–6.78 (d, J = 8, 1H aromatic, 4-H); 6.87–6.90 (d, J = 8, 2H aromatic, 2-H); 7.13–7.21 (m, 2H aromatic, 3-H, 3-H); 7.13–7.21 (br, 1H*, NH); 7.38 (t, J = 7, 1H aromatic, Py4-H); 8.12–8.13 (d, J = 5, 1H aromatic, Py6-H); MS: m/z (%) 615 (M+, 14), 229 (100), 109 (96); Anal. Calcd. for C38H52N8O·3C4H6O6: C, 69.0; H, 8.04; N, 17.70. Found: C, 68.80; H, 8.27; N, 17.70.

N-Cyano-N’-[4-[N-[2-[N-(4-methoxybenzyl)-N-(2-pyridyl)amino]ethyl]-N-methylamino]butyl]-N”-[3-[piperidinomethyl]phenoxy]propyl]guanidine (18). Yield: 47%, mp: 106–108 °C; 1H-NMR (CDCl3): δ ppm = 1.44 (m, 4H, PipH); 1.59 (m, 6H, PipH); 1.91 (m, 2H, CH2); 2.50 (s, 3H, N-CH3); 2.64 (m, 4H, PipH); 2.86 (m, 2H, CH2-NCH3); 3.11 (m, 2H, CH2-NCH3); 3.28 (m, 4H, CH2-NH, Ph-CH2-Pip); 3.44 (m, 2H, CH2-NH); 3.71 (s, 3H, Ph-OCH3); 3.75 (m, 2H, CH2-N), 3.97 (m, 2H, Ph-O-CH2); 4.12 (s, 6H, TA); 4.65 (s, 2H, Ph-CH2-N); 6.00 (br, 6H*, TA-OH); 6.55–6.58 (m, 2H aromatic, Py5-H, Py3-H); 6.86–6.88 (m, 3H aromatic, 4-H, 2-H); 6.86–6.88 (br, 4H*, 3 (H-(N+)), NH); 6.92–7.00 (m, 2H aromatic, 2-H); 7.06 (br, 1H*, NH); 7.13 (m, 2H aromatic, 3-H); 7.26 (m, 1H aromatic, 3-H); 7.43–7.46 (d, J = 7, 1H aromatic, Py4-H); 8.08 (m, 1H aromatic, Py6-H); 10.50 (br, 3H*, TA-COOH); MS: m/z (%) 641 (M+, 1), 241 (7), 121 (100); Anal. Calcd. for C37H52N8O2·3C4H6O6: C, 51.60; H, 6.29; N, 17.70. Found: C, 52.01; H, 6.47; N, 17.30.
N-Cyano-N'-[4-[N-[2-[N-(4-fluorobenzyl)-N-(2-pyridyl)amino]ethyl]-N-methylamino]butyl]-N''-[3-[3-(piperidinomethyl)phenoxy]propyl]guanidine (19). Yield: 48%, mp: 84–86 °C; $^1$H-NMR (CDCl$_3$): δ ppm = 1.37 (m, 2H, CH$_2$); 1.46 (m, 2H, CH$_2$); (see 15); MS: m/z (%) 629 (M$^+$, 5), 229 (84), 109 (100); Anal. Calcd. for C$_{36}$H$_{49}$FN$_8$O·3C$_4$H$_6$O$_6$: C, 53.90; H, 6.73; N, 10.40. Found: C, 51.30; H, 6.46; N, 9.97.

N-Cyano-N'-[4-[N-[2-[N-(4-methoxybenzyl)-N-(2-pyridyl)amino]ethyl]-N-methylamino]pentyl]-N''-[3-[3-(piperidinomethyl)phenoxy]propyl]guanidine (20). Yield: 17%, mp: 86–88 °C; $^1$H-NMR (CDCl$_3$): δ ppm = 1.22 (m, 4H, 2(CH$_2$)); (see 17); MS: m/z (%) 643 (M$^+$, 7), 229 (54), 154 (100); Anal. Calcd. for C$_{37}$H$_{51}$FN$_8$O·3C$_4$H$_6$O$_6$: C, 53.80; H, 6.36; N, 10.30. Found: C, 54.30; H, 6.83; N, 10.30.

N-Cyano-N'-[6-[N-[2-[N-(4-methoxybenzyl)-N-(2-pyridyl)amino]ethyl]-N-methylamino]hexyl]-N''-[3-[3-(piperidinomethyl)phenoxy]propyl]guanidine (21). Yield: 66%, mp: 79–81 °C; $^1$H-NMR (CDCl$_3$): δ ppm = 1.23–1.25 (m, 4H, 2(CH$_2$)); 1.42–1.43 (m, 4H, 2(CH$_2$)); (see 14); MS: m/z (%) 669 (M$^+$, 5), 121 (100); Anal. Calcd. for C$_{39}$H$_{56}$N$_8$O$_2$·3C$_4$H$_6$O$_6$: C, 54.70; H, 6.66; N, 10.10. Found: C, 54.60; H, 7.01; N, 9.79.

N-Cyano-N'-[7-[N-[2-[N-(4-fluorobenzyl)-N-(2-pyridyl)amino]ethyl]-N-methylamino]heptyl]-N''-[3-[3-(piperidinomethyl)phenoxy]propyl]guanidine (22). Yield: 35%, mp: >58 °C decomposed; $^1$H-NMR (CDCl$_3$): δ ppm = 1.68-1.75 (m, 2H, CH$_2$); (see 17); MS: m/z (%) 629 (M$^+$, 18), 229 (98), 109 (100); Anal. Calcd. for C$_{36}$H$_{49}$FN$_8$O·1.5C$_{20}$H$_{18}$O$_8$: C, 64.60; H, 6.36; N, 9.13. Found: C, 64.40; H, 6.51; N, 8.77.

N-Cyano-N'-[2-[[(2-guanidino-4-thiazolyl)methyl]thio]ethyl]-N''-[2-[N-[2-[N-(4-methoxybenzyl)-N-(2-pyridyl)amino]ethyl]-N-methylamino]ethyl]guanidine (25). Yield: 58%, mp: 56–58 °C; $^1$H-NMR (CDCl$_3$): δ ppm = 2.23 (s, 3H, N-CH$_3$); 2.50 (m, 4H, CH$_2$-S, CH$_2$-NCH$_3$); 2.56 (m, 2H, CH$_2$-NCH$_3$); 3.16–3.17 (m, 2H, CH$_2$-NH); 3.26–3.28 (m, 2H, CH$_2$-NH); 3.37 (s, 2H, Thi-CH$_2$-S); 3.58 (m, 2H, CH$_2$-N); 3.70 (s, 3H, Ph-OCH$_3$); 4.66 (s, 2H, Ph-CH$_2$-N); 6.45 (s, 1H aromatic, Thi5-H); 6.50–6.54 (m, 2H aromatic, Py5-H, Py3-H); 6.84–6.87 (m, 2H aromatic, 2-H); 6.84–6.87 (br, 2H*, 2(NH)); 6.94 (br, 4H*, NC(NH$_2$)$_2$); 7.14 (d, J = 8, 2H aromatic, 3-H); 7.41 (m, 1H aromatic, Py4-H); 8.05–8.07 (d, J = 4, 1H aromatic, Py6-H); MS: m/z (%) 596 (M$^+$, 2), 121 (100); Anal. Calcd. for C$_{27}$H$_{37}$N$_{11}$OS$_2$·1.5C$_2$H$_2$O$_4$: C, 54.30; H, 6.75; N, 24.0. Found: C, 54.21; H, 6.63; N, 24.11.
N-Cyano-N’-[2-[(2-guanidino-4-thiazolyl)methyl]thioethyl]N’’-[2-[N-2-[N-(4-fluoro-benzyl)-N-(2-pyridyl)amino]ethyl]-N-methylamino]ethyl]guanidine (26). Yield: 50%, mp: >109 °C decomposed; ¹H-NMR (CDCl₃): δ ppm = 2.23 (s, 3H, N-CH₃); 2.50 (m, 2H, CH₂-S); 2.55 (m, 4H, 2(Ch₂-NCH₃)); 3.15 (m, 2H, CH₂-NH); 3.27 (m, 2H, CH₂-NH); 3.58 (m, 4H, Thi-CH₂-S, CH₂-N); 4.72 (s, 2H, Ph-CH₂-N); 6.46 (s, 1H, Thi5-H); 6.51–6.54 (m, 2H aromatic, Py5-H, Py3-H); 6.80 (br, 6H*, 2(NH), NC(NH₂)₂); 7.11 (t, J=9, 2H aromatic, 2-H); 7.22–7.24 (d, J=6, 2H aromatic, 3-H); 7.44 (m, 1H aromatic, Py4-H); 8.06 (d, 1H aromatic, Py6-H); MS: m/z (%) 584 (M+, 3), 121 (100); Anal. Calcd. for C₂₆H₃₄FN₁₁S₂·2C₄H₆O₆·1.5H₂O: C, 44.80; H, 5.42; N, 16.90. Found: C, 44.61; H, 4.91; N, 17.11.

N-Cyano-N’-[2-[(2-guanidino-4-thiazolyl)methyl]thioethyl]N’’-[3-[N-[2-[N-(4-methoxybenzyl)-N-(2-pyridyl)amino]ethyl]-N-methylamino]propyl]guanidine (27). Yield: 45%, mp: 54–56 °C; ¹H-NMR (CDCl₃): δ ppm = 1.66 (m, 2H, CH₂); 2.26 (s, 3H, N-CH₃); 2.48 (m, 2H, CH₂-S); 2.58 (t, J=7, 2H, CH₂-NCH₃); 2.68 (t, J=7, 2H, CH₂-NCH₃); 3.21 (m, 2H, CH₂-NH); 3.29 (m, 2H, CH₂-NH); 3.58 (s, 2H, Thi-CH₂-S); 3.72 (t, J=8, 2H, CH₂-N); 3.76 (s, 3H, Ph-OCH₃); 4.60 (s, 2H, Ph-CH₂-N); 5.74 (br, 2H*, 2(NH)); 6.33 (S, 1H aromatic, Thi5-H); 6.43–6.44 (d, J=8.5, 2H aromatic, Py5-H, Py3-H); 6.54 (br, 4H*, NC(NH₂)₂); 6.81–6.84 (m, 2H aromatic, 2-H); 7.10–7.13 (d, J=8.5, 2H aromatic, 3-H); 7.34–7.39 (m, 1H aromatic, Py4-H); 8.11–8.12 (d, J=3, 1H aromatic, Py6-H); MS: m/z (%) 624 (M⁺, 1), 121 (100); Anal. Calcd. for C₂₈H₃₉N₁₁OS₂·H₂O: C, 53.60; H, 6.58; N, 24.50. Found: C, 53.81; H, 6.43; N, 24.41.

N-Cyano-N’-[3-[N-2-[N-(4-fluorobenzyl)-N-(2-pyridyl)amino]ethyl]-N-methylamino]propyl]guanidine (28). Yield: 35%, mp: oil; ¹H-NMR (CDCl₃): δ ppm = 1.67 (m, 2H, CH₂); 2.27 (s, 3H, N-CH₃); 2.49 (t, 2H, CH₂-S); 2.58 (t, 2H, CH₂, NCH₃); 2.73 (t, 2H, CH₂-NCH₃); 3.21 (m, 2H, CH₂-NH); 3.32 (m, 2H, CH₂-NH); 3.60 (s, 2H, Thi-CH₂-S); 3.73 (m, 2H, CH₂-N); 4.66 (s, 2H, Ph-CH₂-N); 6.35 (s, 1H aromatic, Thi5-H); 6.41–6.43 (d, J=9, 1H aromatic, Py5-H); 6.57 (t, J=5, 1H aromatic, Py3-H); 6.80 (br, 6H*, 2(NH), NC(NH₂)₂); 6.99 (t, J=8.5, 2H aromatic, 2-H); 7.17 (m, 2H aromatic, 3H); 7.37–7.39 (m, 1H aromatic, Py4-H); 8.14 (d, 1H aromatic, Py6-H); MS: m/z (%) 598 (M⁺, 2), 121 (100); Anal. Calcd. for C₂₇H₃₉N₁₁OS₂·1.5H₂O: C, 53.60; H, 6.58; N, 24.50. Found: C, 53.81; H, 6.43; N, 24.40.

N-Cyano-N’-[4-[N-2-[N-(4-methoxybenzyl)-N-(2-pyridyl)amino]ethyl]-N-methylamino]butyl]guanidine (29). Yield: 60%, mp: hygroscopic foam; ¹H-NMR (CDCl₃): δ ppm = 1.36–1.44 (m, 4H, 2(Ch₂)); (see 25); MS: m/z (%) 624 (M⁺, 1), 121 (100); Anal. Calcd. for C₂₉H₄₁N₁₁OS₂·1.5H₂O: C, 53.50; H, 6.81; N, 23.70. Found: C, 53.42; H, 6.51; N, 24.01.

N-Cyano-N’-[4-[N-2-[N-(4-fluorobenzyl)-N-(2-pyridyl)amino]ethyl]-N-methylamino]butyl]guanidine (30). Yield: 60%, mp: >137 °C decomposed; ¹H-NMR (CDCl₃): δ ppm = 1.49–1.58 (m, 2H, CH₂); (see 28); MS: m/z (%) 612 (M⁺, 2), 229 (100), 109 (38); Anal. Calcd. for C₂₉H₃₈FN₁₁S₂·1.5C₂₀H₁₈O₈·2H₂O: C, 56.80; H, 5.66; N, 12.60. Found: C, 56.40; H, 5.30; N, 12.51.

N-Cyano-N’-[5-[N-2-[N-(4-fluorobenzyl)-N-(2-pyridyl)amino]ethyl]-N-methylamino]pentyl]guanidine (31). Yield: 29%, mp: 103–105 °C; ¹H-NMR
(CDCl₃): δ ppm = 1.23 (m, 2H, CH₂); 1.35 (m, 4H, 2(CH₂)); (see 26); MS: m/z (%) 626 (M⁺, 3), 154 (100); Anal. Calcd. for C₂₉H₄₀FN₁₁S₂·3C₄H₆O₆·0.5H₂O: C, 45.40; H, 5.48; N, 14.20. Found: C, 45.12; H, 5.32; N, 14.19.

N-Cyano-N'[(2-guanidino-4-thiazolyl)methyl]thioethyl]N'-[6-[N-[2-(4-methoxybenzyl)-N-(2-pyridyl)amino]ethyl]N-methylamino]hexyl]guanidine (32). Yield: 29%, mp: >52 °C decomp.; ¹H-NMR (CDCl₃): δ ppm = 1.27 (m, 4H, 2(CH₂)); 1.42 (m, 2H, CH₂); (see 27); MS: m/z (%) 652 (M⁺, 12), 121 (100); Anal. Calcd. for C₃₁H₄₅N₁₁OS₂·0.5H₂O: C, 56.31; H, 7.01; N, 23.30. Found: C, 56.42; H, 7.17; N, 23.31.

N-Cyano-N'[(2-guanidino-4-thiazolyl)methyl]thioethyl]N'-[6-[N-[2-(4-fluorobenzyl)-N-(2-pyridyl)amino]ethyl]N-methylamino]hexyl]N'-[2-[2-guanidino-4-thiazolyl]methyl]thioethyl]guanidine (33). Yield: 61%, mp: 75–77 °C decomp.; ¹H-NMR (CDCl₃): δ ppm = 1.26 (m, 4H, 2(CH₂)); 1.43 (m, 2H, CH₂); (see 28); MS: m/z (%) 639 (M⁺, 9), 229 (100), 109 (88); Anal. Calcd. for C₃₀H₄₂FN₁₁S₂·3C₄H₆O₆·0.25H₂O: C, 46.10; H, 5.57; N, 14.10. Found: C, 45.81; H, 5.62; N, 14.62.

N-Cyano-N'[(2-guanidino-4-thiazolyl)methyl]thioethyl]N'-[7-[N-[2-[N-(4-fluorobenzyl)-N-(2-pyridyl)amino]ethyl]-N-methylamino]heptyl]N'-[2-[2-guanidino-4-thiazolyl]methyl]thioethyl]guanidine (34). Yield: 24%, mp: 118–120 °C; ¹H-NMR (CDCl₃): δ ppm = 1.24 (m, 6H, 3(CH₂)); 1.42 (m, 2H, CH₂); (see 28); MS: m/z (%) 654 (M⁺, 2), 91 (100); Anal. Calcd. for C₃₁H₄₄N₁₁S₂·1.5C₂₀H₁₈O₈·2H₂O: C, 57.61; H, 5.51; N, 12.42.

N-Cyano-N'[(3-[N-[2-(4-methoxybenzyl)-N-(2-pyridyl)amino]ethyl]-N-methylamino]propyl]N'-[2-[5-(dimethylaminomethyl)furfuryl]thio]ethyl]guanidine (35). Yield: 38%, mp: 74–76 °C; ¹H-NMR (CDCl₃): δ ppm = 1.68 (q, 2H, CH₂); 2.23 (s, 6H, N(CH₃)₂); 2.28 (s, 3H, N-CH₃); 2.49 (t, 2H, CH₂-S); 2.56–2.65 (m, 4H, 2(CH₂-NCH₃)); 3.23–3.30 (m, 4H, 2(CH₂-NH)); 3.40 (s, 2H, Fur-CH₂-S); 3.67 (s, 2H, Fur-CH₂-N); 3.75 (t, J = 8, 2H, CH₂-N); 3.78 (s, 3H, Ph-CH₂CH₃); 4.63 (s, 2H, Ph-CH₂-N); 6.11–6.12 (q, J = 4, 2H aromatic, Fur3-H, Fur4-H); 6.44–6.47 (d, J = 8.5, 1H aromatic, Py5-H); 6.55 (m, 1H aromatic, Py3-H); 6.55 (br, 1H*, NH); 6.83–6.85 (d, J = 8.5, 2H aromatic, 2-H); 6.83–6.85 (br, 1H*); 7.12–7.15 (d, J = 8.5, 2H aromatic, 3-H); 7.38 (m, 1H aromatic, Py4-H); 8.12–8.13 (d, J = 3, 1H aromatic, Py6-H); MS: m/z (%) 595 (M⁺, 4), 121 (100); Anal. Calcd. for C₃₃H₄₄N₁₀O₂S·3C₄H₆O₆·0.5H₂O: C, 49.10; H, 6.03; N, 10.71. Found: C, 48.71; H, 6.26; N, 11.22.

3.2. Pharmacology

3.2.1. Histamine H₁ Receptor Antagonist Activity on the Isolated Guinea Pig Ileum

H₁ receptor antagonist activity was determined from isotonically recorded (load 10 mN) cumulative concentration-response curves as described using histamine dihydrochloride (0.01–3 µM) as the reference agonist [31]. Whole segments of proximal ileum were mounted in an organ bath containing 20 mL of Tyrode solution ([mM] NaCl 137, KCl 2.7, NaH₂PO₄ 4.2, NaHCO₃ 11.9, CaCl₂ 1.8, MgCl₂ 1.0, d-glucose 5.6) containing 0.1 µM atropine, aerated with 95% O₂/5% CO₂, bath temperature 37 °C. The compounds were tested at different concentrations with at least four experiments for each concentration. Determination of H₁ receptor antagonism at low concentrations (≤10⁻⁸ M) of the
potential antagonists required a longer incubation period up to 20 min in order to achieve equilibrium states; at higher concentrations periods of 10–15 min were sufficient. $pK_B$ values (mean of at least three independent experiments) were calculated from the expression $pK_B = -\log [\text{antagonist}] + \log (\text{concentration ratio} - 1)$ as the compounds produced a dose-dependent depression of the concentration-response curves [32].

3.2.2. Histamine H$_2$ Receptor Antagonist Activity on the Isolated Guinea Pig Right Atrium

The investigations for H$_2$ receptor antagonism followed the procedure described by Black et al. [33] with minor modifications. In brief, male guinea pigs (350–400 g) were killed by a blow on the head and exsanguinated. Right atria were rapidly removed, attached to a tissue holder in an organ bath (32.5 °C) containing 20 mL of Krebs-Henseleit solution, containing [mM] NaCl 118, NaHCO$_3$ 25, KCl 4.7, KH$_2$PO$_4$ 1.2, CaCl$_2$ 2.5, MgSO$_4$ 1.6, glucose 6.2, gassed with 95% O$_2$/5% CO$_2$, bath temperature 32.5 °C. The antagonistic potency was determined from isometrically recorded cumulative concentration-response curves using histamine dihydrochloride (0.1–10 µM) as the reference substance [34]. The incubation period was generally 30 min for the antagonists. For pharmacological screening, 2–5 experiments were carried out for each antagonist at one or two concentrations (Table 1) and $pK_B$ values were calculated as above.

4. Conclusions

Starting from lead compound 1 according to the working hypothesis that the incorporation of a mepyramine-like structure linked via a six-membered-spacer with a cyanoguanidine instead of urea as a polar group and combined with a tiotidine-like partial structure, significantly improved H$_1$R antagonist activity and restored pronounced H$_2$R antagonism. However, the affinity at both receptors should be further increased with respect to the development of combined H$_1$R/H$_2$R antihistamine as potential pharmacotherapeutic agents. Generally, pharmacological hybrids harbour the potential of improving the pharmacokinetic properties compared to single drugs with different half lives. Further progress in structural optimization seems possible through selection of other H$_1$R and H$_2$R pharmacophoric moieties known to possess histamine receptor subtype affinity. Nevertheless, this has to be proven experimentally, because the mutual affinity-conferring or -enhancing contribution of the respective substructures cannot be predicted on the basis of the structure-activity relationships available so far. Different routes of administration are conceivable, for example, topical applications of combined H$_1$R/H$_2$R antagonists in dermatology or systemic administration for premedication in anaesthesia and surgery or for the prevention of hypersensitivity reactions to drug treatment, e.g., in cancer chemotherapy. This has to be taken into consideration with respect to optimization of the physicochemical properties.

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Conflicts of Interest

The authors declare no conflict of interest.

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*Sample Availability:* Samples of the compounds *25* and *32* are available from the authors.

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