# Synthesis of 1,2,4-triazole Derivatives: Binding Properties on Endothelin Receptors[§]

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**Abstract:** In the present study we describe the synthesis of a new series of 1,2,4-triazoles: [3-(arylmethyl)thio-5-aryl-4*H*-[1,2,4]triazol-4-yl]acetic acids **5a-g**, [5-(arylmethyl)thio-3-aryl-1*H*-[1,2,4]triazol-1-yl]acetic acids **8a-d**, and [3-(arylmethyl)thio-5-aryl-1*H*-[1,2,4]triazol-1-yl] acetic acids **9a-d**. These compounds were tested in binding assays to evaluate their ability as ligands for human $ET_A$ and $ET_B$ receptors stably expressed in CHO cells; some of the tested compounds showed affinity in the micromolar range.

**Key Words:** Hypertension, endothelins, ET receptors, binding assays, 1,2,4-triazoles, regioisomers.

## INTRODUCTION

The endothelins (ETs) constitute a family of 21-amino acid isopeptides (ET-1, ET-2, ET-3, and ET-4), isolated from porcine aortic endothelial cells by Yanagisawa and coworkers in 1988, with ET-1 being one of the most potent vasoconstrictors identified to date [1]. The ETs produce a variety of physiological activities such as modulation of vascular tone, cell proliferation and production of hormones [2]. The ETs exert their effects *via* activation of two distinct G protein-coupled receptor subtypes termed $ET_A$ and $ET_B$. Both receptors are found in a wide variety of tissues in particular vascular smooth muscles and endothelial cells. Pharmacological studies indicate that elevated levels of plasma concentration of ETs were observed in several disease states where excessive vasoconstriction or smooth muscle proliferation play a role. These include systemic hypertension, pulmonary hypertension, acute myocardial infarction, congestive heart failure, renal failure, cancer, and atherosclerosis. Therefore ET receptor antagonists may constitute a novel class of agents for the treatment of the above-mentioned pathological conditions [1-6].

Early developments of ET antagonists were initiated using peptidic compounds, including BQ-123 [7] and BQ-788 [8]; at the present, non-peptidic ET antagonists, including commercially available Bosentan [9], are known. Some are selective antagonists for $ET_A$ receptors, and others act on both $ET_A$ and $ET_B$ receptors. Although it remains unclear which type of antagonists, selective $ET_A$ or mixed $ET_A/ET_B$, is more suitable for clinical use [10], these findings prompted us to develop novel non-peptidic ET receptor ligands. From a careful survey of the chemical structure of non-peptidic ET receptor ligands, some common chemical features emerged: (i) an acidic function represented by a carboxyl acid group or a sulfonamide moiety, (ii) one or more lipophilic aromatic rings, usually substituted with electron releasing groups such as methoxy or methylenedioxy, (iii) a rigid scaffold which supports the above-mentioned moieties; the acidic function is preferentially located between two lipophilic aromatic portions [6, 11-14]. Chemical structures of compounds **1**, **2**, and **3**, representative of potent non-peptidic ET receptor ligands ($ET_A$ $IC_{50}$ = 1.5, 0.086, and 0.028 μM; $ET_B$ $IC_{50}$ = 3.3, 7.4, and 0.040 μM, for compounds **1**, **2**, and **3**, respectively), are reported in Fig. (**1**) [13,14]. In compounds **1** and **2** the rigid scaffold is a bicyclic

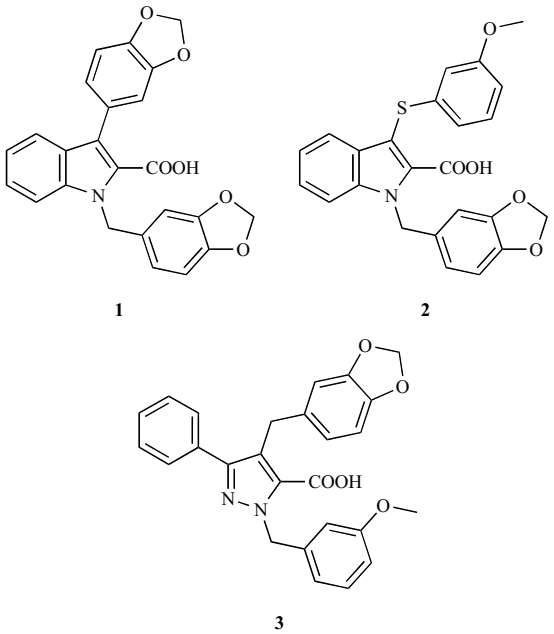


**Fig. (1).** Structures of ET receptor ligands **1-3**.

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indole which bears the carboxyl function at the C-2, between two lipophilic aromatic residues substituted with electron releasing groups such as methoxy or methylenedioxy. These aromatic residues can be directly or indirectly linked to the indole C-3. In compound **3**, which belongs to a series of derivatives that contain a central monocyclic pyrazol-5-carboxyl acid as scaffold, the two required aromatic moieties substituted with electron releasing groups are both linked at pyrazole ring through a methylene chain.

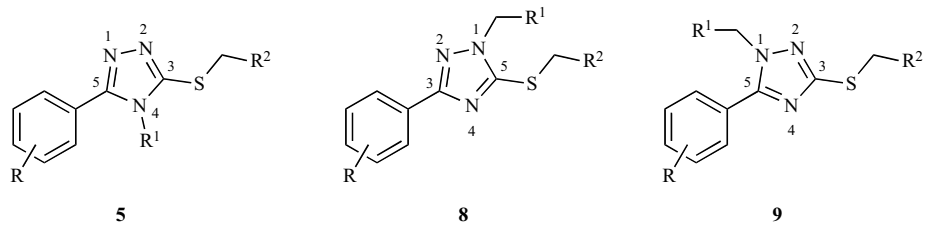
As part of our efforts in the design of novel ET receptor ligands belonging to different chemical classes [15-17], in the present study we describe the synthesis and binding assays of a new series of [1,2,4]triazoles. The [1,2,4]triazole nucleus was chosen as rigid scaffold because it well mimicks the pentacyclic pyrazole nucleus of compound **3**; in addition, it was possible to modify all the positions of this five-member ring. The carboxyl acid group was linked, by means of a methylene moiety, at the different nitrogen atoms of the triazole nucleus, to give the three series of regioisomers, depicted in Fig. (**2**), [3-(arylmethyl)thio-5-aryl-4*H*-[1,2,4]triazol-4-yl]acetic acids **5**, [5-(arylmethyl)thio-3-aryl-1*H*-[1,2,4]triazol-1-yl]acetic acids **8**, [3-(aryl-methyl)thio-5-aryl-1*H*-[1,2,4]triazol-1-yl]acetic acids **9**. The two substituted aromatic rings were, the first directly linked at the 5- or 3-position of triazole nucleus, whereas the second was fixed at the 3- or 5-position of triazole ring by means of a methylthio chain, in order of obtaining compounds which can better adjust their conformation for ET receptor binding. Among the three series of regioisomers **5**, **8**, and **9**, compounds which better respond to the chemical features emerged from literature, in particular the position of the carboxyl group *versus* the aromatic rings, are the regioisomers of the series **5**. In fact, in this series, the acidic function is located between the two aromatic portions. The other regioisomers were synthesized and tested to extend structure-affinity relationships study on this new class of triazole derivatives.

Synthesized compounds were tested in binding assays to evaluate their ability as ligands for human endothelin A ($ET_{Ah}$) and human endothelin B ($ET_{Bh}$) receptors.

## CHEMISTRY

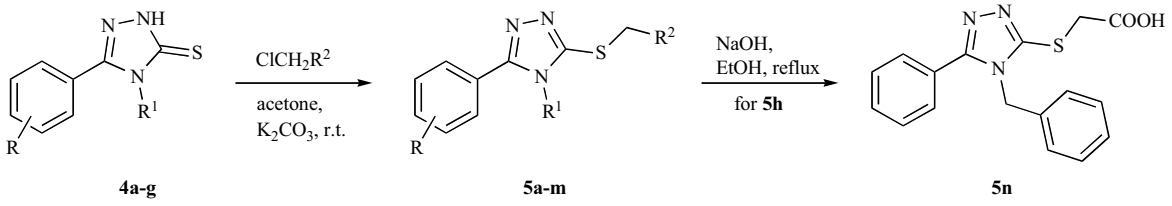
General procedures for the synthesis of the three series of regioisomers **5**, **8**, and **9** are depicted in Schemes **1**, **2** and **3**.

Compounds **5a-g**, were obtained by the unambigous synthesis described in Scheme **1**; briefly, [5-aryl-3-mercapto-4*H*-[1,2,4]triazol-4-yl]acetic acids **4a-c**, prepared by literature procedures [18], were readly S-alkylated using various arylalkylchlorides, at room temperature, in the presence of $K_2CO_3$ and acetone. Alkylation of triazole **4d** with ethyl chloroacetate afforded ester **5h** which was next hydrolyzed with 5% NaOH in ethanolic medium to give desired acid **5n**.



**5** **8** **9**

**Fig. (2).** Structures of target compounds of the three series **5**, **8**, and **9**.

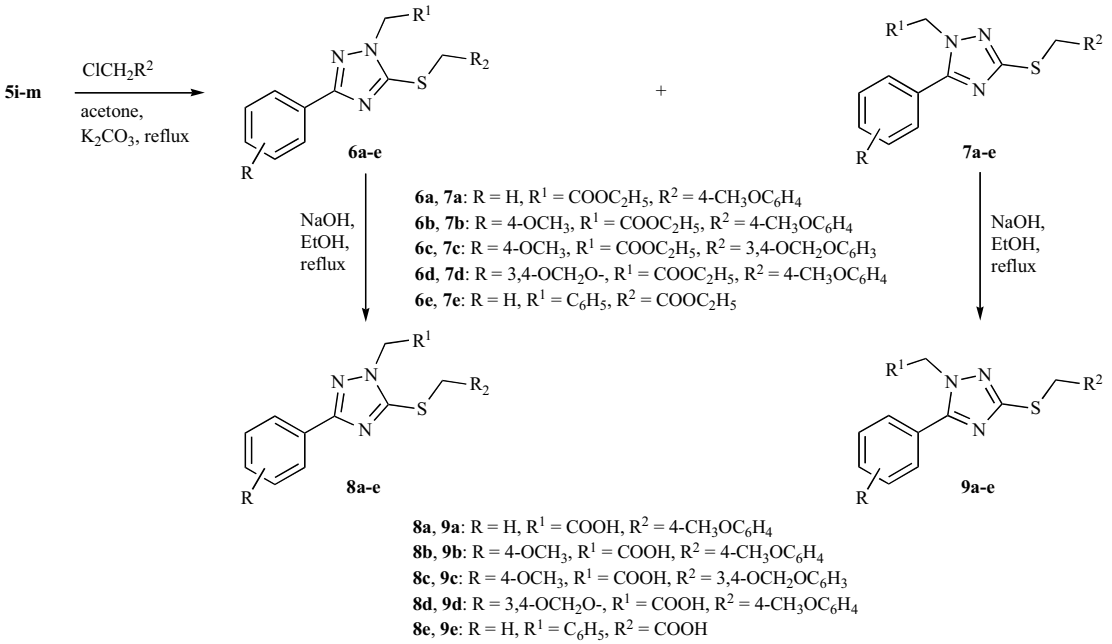


**4a-g** **5a-m** **5n**

**4a**: R = H, $R^1$ = $CH_2COOH$
**4b**: R = 4-$OCH_3$, $R^1$ = $CH_2COOH$
**4c**: R = 3,4-$OCH_2O$-, $R^1$ = $CH_2COOH$
**4d**: R = H, $R^1$ = $CH_2C_6H_5$
**4e**: R = H, $R^1$ = H
**4f**: R = 4-$OCH_3$, $R^1$ = H
**4g**: R = 3,4-$OCH_2O$-, $R^1$ = H

**5a**: R = H, $R^1$ = $CH_2COOH$, $R^2$ = 4-$CH_3OC_6H_4$
**5b**: R = 4-$OCH_3$, $R^1$ = $CH_2COOH$, $R^2$ = 4-$CH_3OC_6H_4$
**5c**: R = 4-$OCH_3$, $R^1$ = $CH_2COOH$, $R^2$ = 3,4-$OCH_2OC_6H_3$
**5d**: R = 3,4-$OCH_2O$-, $R^1$ = $CH_2COOH$, $R^2$ = 4-$CH_3OC_6H_4$
**5e**: R = 3,4-$OCH_2O$-, $R^1$ = $CH_2COOH$, $R^2$ = 2-$CH_3OC_6H_4$
**5f**: R = 3,4-$OCH_2O$-, $R^1$ = $CH_2COOH$, $R^2$ = 3-$CH_3OC_6H_4$
**5g**: R = 3,4-$OCH_2O$-, $R^1$ = $CH_2COOH$, $R^2$ = 4-$CH_3SC_6H_4$
**5h**: R = H, $R^1$ = $CH_2C_6H_5$, $R^2$ = $COOC_2H_5$
**5i**: R = H, $R^1$ = H, $R^2$ = 4-$CH_3OC_6H_4$
**5j**: R = 4-$OCH_3$, $R^1$ = H, $R^2$ = 4-$CH_3OC_6H_4$
**5k**: R = 4-$OCH_3$, $R^1$ = H, $R^2$ = 3,4-$OCH_2OC_6H_3$
**5l**: R = 3,4-$OCH_2O$-, $R^1$ = H, $R^2$ = 4-$CH_3OC_6H_4$
**5m**: R = H, $R^1$ = H, $R^2$ = $COOC_2H_5$

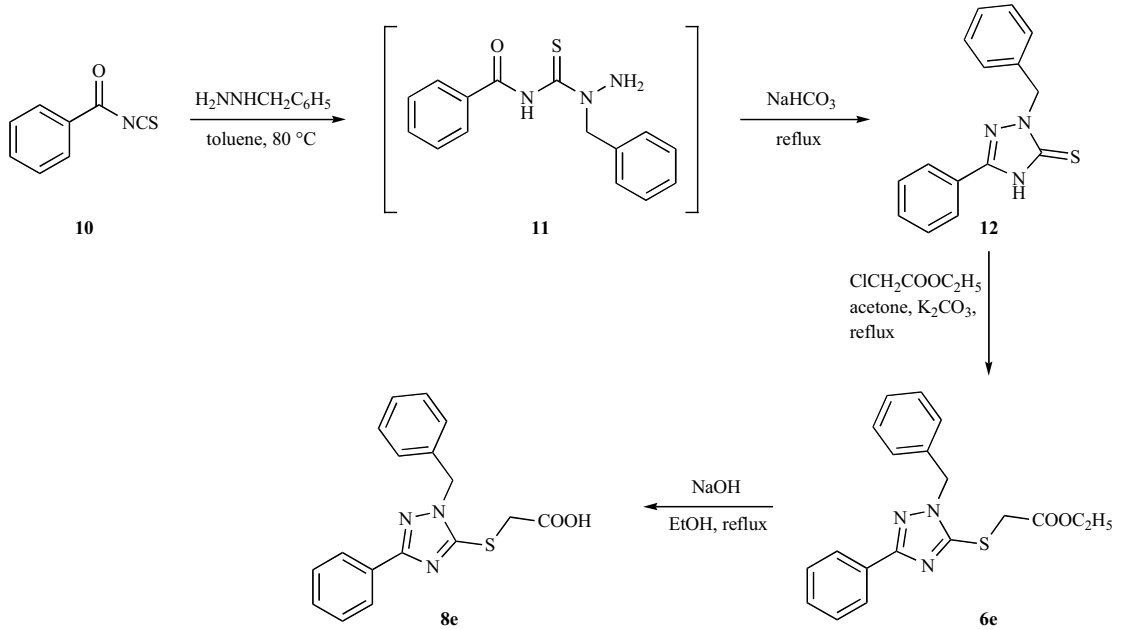
**Scheme 1.** Synthesis of target compounds **5a-g**, **5n**, and intermediates **5h-m**.




**Scheme 2.** Synthesis of target compounds **8a-e** and **9a-e**.

Moreover, 5-aryl-2,4-dihydro-3*H*-[1,2,4]triazole-3-thiones **4e-g** [19-20] were S-alkylated with arylalkylchlorides or ethyl chloroacetate, under the same above conditions, to give triazoles **5i-m** which were used as starting materials into the next steps.

Isomers **8a-e** and **9a-e**, were both obtained in one step as described in Scheme **2**; briefly, 3-(arylmethyl)thio-5-aryl-4*H*-[1,2,4]-triazoles **5i-m** were N-alkylated using ethyl chloroacetate or benzyl chloride in the presence of $K_2CO_3$ and acetone, at reflux; the obtained regioisomeric esters **6a-e** and **7a-e** were separated by flash chromatography; usually, the first collected esters **6a-e** were obtained in higher yields than derivatives **7a-e**. Hydrolysis of esters **6a-e** and **7a-e** in alkaline medium gave corresponding acids **8a-e** and **9a-e**. All the synthesized compounds had satisfactory elemental analysis and their IR and $^1$H NMR spectra were consistent with the proposed structures. In particular, $^1$H NMR spectra differentiated clearly between **8** and **9** regioisomers. A sum-




**Scheme 3.** Synthesis of target compound **8e**.

mary of the more representative $^1$H NMR signals of regioisomers **5**, **8**, and **9**, is showed in Table **1**.

Among compounds described in Scheme **2**, the pair of regioisomers **8e** and **9e** was prepared for an analytical purpose, mainly to confirm the proposed structure of regioisomers of series **8** and **9**. Thus, in addition to the synthetic pathway shown in Scheme **2**, compound **8e** was unequivocally prepared as described in Scheme **3**. More specifically, condensation of benzoyl isothiocyanate **10** with benzyl hydrazine in toluene gave at 80 °C, *via* intermediate **11**, the desired 2-phenylmethyl-5-phenyl-2,4-dihydro-3*H*-[1,2,4] triazole-3-thione **12** [21, 22]. S-alkylation of **12** with ethyl chloroacetate, in the presence of $K_2CO_3$ and acetone at reflux, gave ester **6e** which was hydrolyzed with 5% NaOH in ethanol to give desired **8e**.

Like compound **5n**, **8e** and **9e** bear the acetic moiety at the exocyclic sulphur atom and the phenylmethyl substituent at one of the nitrogen atoms of the triazole ring. All three regioisomers clearly showed discrete IR and $^1$H NMR spectra. In the analysis of $^1$H NMR spectra (see Table **1** for more representative signals) it is noteworthy that the singlet signal for $NCH_2$ methylene hydrogens is shifted downfield in the sequence **5n**, **8e**, **9e** (δ 5.28, 5.38, and 5.44, respectively). Ortho hydrogens of the phenyl ring directly linked at the 3- (or 5-) position of the triazole nucleus give the most downfield signals among aromatic hydrogens. However, in **8e** this signal is an isolated multiplet at δ 7.92-7.97 whereas signals for analogous hydrogens in **5n** and in **9e** are located upfield and are partly overlapping the others. Since regioisomers **5n** and **8e** were prepared by univocal synthetic pathways (Scheme **1** and Scheme **3**, respectively), the structure of the third isomer **9e** was undoubtedly inferred.

Regioisomers **5a**, **8a**, and **9a**, having a (4-methoxyphenyl)methyl moiety at the exocyclic sulphur atom, bear an acetic group at one of the nitrogens of the triazole ring. As in the case of **5n**, **5a** was obtained by means of an univocal synthesis (Scheme **1**). Structures of remaining isomers **8a** and **9a** can be deduced from the following observations: (i) **8a** was obtained by hydrolysis of **6a** which was the first eluted ethyl ester of the isomeric pair **6a**/**7a**. Analogously, **8e** had been obtained by hydrolysis of the first eluted ethyl ester **6e** of the pair **6e**/**7e**; (ii) in $^1$H NMR spectrum of **8a**, the singlet for the $NCH_2$ methylene hydrogens is located upfield with respect to the corrresponding signal in **9a**. The same is observed in **8e** when compared to **9e** (Table **1**); (iii) ortho hydrogens of the phenyl ring linked to triazole give a multiplet which in the $^1$H NMR spectrum of **8a** is placed downfiled to the corresponding signal in **9a**, as previously observed in **8e** *versus* **9e** (Table **1**). On these bases, we proposed that in **8a** the triazole nitrogen bearing the substituent is distal to the carbon atom bearing the phenyl ring (3-position), as previously demonstrated in **8e**. Conversely, in **9a** the substituted nitrogen is vicinal to the same carbon of the triazole ring (5-position in this case). Since compounds **8b-d** and **9b-d** are close analogous of **8a** and **9a**, respectively, it is expected that they show the same pattern of substitution.

## RESULTS AND DISCUSSION

Synthesized compounds **5a-g**, **8a-d**, and **9a-d** were tested at maximum concentration of $10^{-5}$ M in binding assays. The

**Table 1. Representative $^1$H NMR Signals (ppm) for the Three Isomeric Series of Compounds 5a-d, 5n, 8a-e, and 9a-e**

| Compound | R | $R^1$ | $R^2$ | $SCH_2$ | $NCH_2$ | Aromatic |
|---|---|---|---|---|---|---|
| **5a** | H | $CH_2COOH$ | 4-$CH_3OC_6H_4$ | 4.31 | 4.64 | 7.51-7.59 (5H) |
| **8a** | H | COOH | 4-$CH_3OC_6H_4$ | 4.43 | 4.92 | 7.98-8.02 (2H) |
| **9a** | H | COOH | 4-$CH_3OC_6H_4$ | 4.32 | 5.06 | 7.65-7.70 (2H) |
| **5b** | 4-$OCH_3$ | $CH_2COOH$ | 4-$CH_3OC_6H_4$ | 4.29 | 4.60 | 7.45-7.49 (2H) |
| **8b** | 4-$OCH_3$ | COOH | 4-$CH_3OC_6H_4$ | 4.41 | 4.87 | 7.90-7.95 (2H) |
| **9b** | 4-$OCH_3$ | COOH | 4-$CH_3OC_6H_4$ | 4.31 | 5.03 | 7.59-7.63 (2H) |
| **5c** | 4-$OCH_3$ | $CH_2COOH$ | 3,4-$OCH_2OC_6H_3$ | 4.26 | 4.64 | 7.46-7.50 (2H) |
| **8c** | 4-$OCH_3$ | COOH | 3,4-$OCH_2OC_6H_3$ | 4.40 | 4.89 | 7.90-7.93 (2H) |
| **9c** | 4-$OCH_3$ | COOH | 3,4-$OCH_2OC_6H_3$ | 4.29 | 5.04 | 7.59-7.65 (2H) |
| **5d** | 3,4-$OCH_2O$- | $CH_2COOH$ | 4-$CH_3OC_6H_4$ | 4.29 | 4.62 | 7.24-7.29 (2H) |
| **8d** | 3,4-$OCH_2O$- | COOH | 4-$CH_3OC_6H_4$ | 4.40 | 4.85 | 7.51-7.56 (2H) |
| **9d** | 3,4-$OCH_2O$- | COOH | 4-$CH_3OC_6H_4$ | 4.31 | 5.06 | 7.30-7.36 (2H) |
| **5n** | H | $CH_2C_6H_5$ | COOH | 4.07 | 5.28 | 7.48-7.57 (5H) |
| **8e** | H | $C_6H_5$ | COOH | 4.15 | 5.38 | 7.92-7.97 (2H) |
| **9e** | H | $C_6H_5$ | COOH | 3.98 | 5.44 | 7.51-7.65 (5H) |

assays were performed on recombinant human ET receptors expressed in CHO-K1 cells using [$^{125}$I]endothelin-1 as radioligand. Compounds BQ-123 [5] and BQ-788 [6] were used as reference substances, being the first a potent and selective ligand for the $ET_{Ah}$ receptors, and the second a potent and selective ligand for the $ET_{Bh}$ receptors. Table **2** summarizes $ET_{Ah}$ and $ET_{Bh}$ binding properties of all synthesized and tested compounds, expressed as percentage of inhibition of specific binding. $K_i$ values are reported only for compounds which showed a percentage of inhibition ≥ 50% at $10^{-5}$ M.

**Table 2. Binding Properties of Compounds 5a-g, 8a-d, and 9a-d**

| Compound | $ET_{Ah}$[a] $10^{-5}$ M[b] | $ET_{Bh}$[a] $10^{-5}$ M[b] $^{125}$I-Endthelin-1 |
|---|---|---|
| **5a** | NA | NA[c] |
| **5b** | NA | NA |
| **5c** | NA | NA |
| **5d** | NA | (10.85 ± 0.87) |
| **5e** | 22 | 12 |
| **5f** | NA | 6 |
| **5g** | 5 | NA |
| **8a** | 11 | (5.98 ± 0.84) |
| **8b** | NA | NA |
| **8c** | 8 | NA |
| **8d** | NA | NA |
| **9a** | NA | NA |
| **9b** | 7 | 15 |
| **9c** | 11 | NA |
| **9d** | NA | NA |
| **BQ-123** | 95 (0.0054 ± 0.0008) | NA |
| **BQ-788** | NA | 100 (0.0071 ± 0.0023) |

[a] Values are the percentage of inhibition of specific binding and are the mean of three separate experiments.

[b] $K_i$ values in μM are reported in brackets.

[c] NA: not active

Among tested compounds, only [5-(1,3-benzodioxol-5-yl)-3-[[(4-methoxyphenyl)methyl]thio]-4*H*-[1,2,4]triazol-4-yl] acetic acid **5d** and [5-[[(4-methoxyphenyl)methyl]thio]-3-phenyl-1*H*-[1,2,4]triazol-1-yl]acetic acid **8a** showed $K_i$ values in the micromolar range (10.85 and 5.98 μM, respectively) on $ET_{Bh}$ receptors and didn't have any affinity for $ET_{Ah}$ receptors.

Despite the low general affinities, some structure-affinity relationships on this new class of triazole derivatives can be discussed. The greater number of compounds endowed with some affinity, prefer $ET_{Bh}$ instead of $ET_{Ah}$ receptors with the only exception of compound **5e**. Although the best affinity value was showed by compound **8a**, the most number of derivatives that show a percentage of inhibition of specific binding, undoubtedly belongs to the series of regioisomers **5**, i.e. that one which better respond to the chemical features emerged from literature. Therefore, the position of the carboxyl group *versus* substituted aromatic rings is critical also for this new class of compounds. Finally, among compounds of series **5**, the presence of a 1,3-benzodioxol-5-yl substituent at the triazole C-5, is essential, as can be seen from the comparison of affinities of compounds **5a-c** *versus* **5d-f**.

In conclusion, although biological results were not completely satisfactory, the preferential binding of compounds **5d** and **8a** on $ET_{Bh}$ receptors may represent a starting point for the development of selective $ET_B$ ligands, since only few ligands for this receptor subtype are reported in the literature. Moreover, even if $ET_B$ receptor antagonist are not probably suitable for clinical use, selective ligands for $ET_B$ receptors could be useful as pharmacological tools to better understand the biological roles of this receptor subtype.

## EXPERIMENTAL SECTION

### Chemistry

Melting points were determined in a Gallenkamp apparatus with a digital thermometer MFB-595 in glass capillary tubes and are uncorrected. Elemental analyses for C, H, N and S were within ± 0.4% of theoretical values and were performed on a Carlo Erba Elemental Analyzer Mod. 1108 apparatus. The IR spectra were recorded in KBr disks on a Perkin Elmer 1600 Series FT-IR spectrometer. $^1$H NMR spectra were recorded at 200 MHz on a Varian Inova Unity 200 spectrometer in DMSO-$d_6$ solution. Chemical shifts are given in δ values (ppm), using tetramethylsilane as the internal standard; coupling constants (J) are given in hertz (Hz). Signal multiplicities are characterized as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad signal). All the synthesized compounds were checked by TLC (aluminum sheet coated with silica gel 60 $F_{254}$, Merck) and visualized by UV (λ = 254 and 366 nm). All chemicals and solvents were reagent grade and were purchased from commercial sources. Compounds **4a-g** were synthesized using literature procedures [18-20]. Synthesis of compound **4c** not previously described is reported as representative example.

#### [5-(1,3-Benzodioxol-5-yl)-3-mercapto-4*H*-[1,2,4]triazol-4-yl]acetic acid 4c

To a solution of 1,3-benzodioxol-5-yl hydrazide [23] (1.32 g, 7.3 mmol) in 20 mL of ethanol, ethyl isothiocyanatoacetate (0.23 mL, 7.5 mmol) was added dropwise. The mixture was stirred at room temperature for 1 h; after this time, the obtained suspension was filtered, washed with a little amount of ethanol and dried to give 2.14 g (90%) of 1,3-benzodioxol-5-yl thiosemicarbazide as a white solid: mp 181-182 °C. $R_f$ = 0.45 (ethyl acetate). $^1$H NMR (DMSO-$d_6$) δ 1.19 (t, J = 7.2 Hz, 3H, $CH_2CH_3$), 4.10 (q, J = 7.2 Hz, 2H, $CH_2CH_3$), 4.18 (s, 2H, $NCH_2$), 6.11 (s, 2H, $OCH_2O$), 7.01-7.05 (m, 1H, aromatic), 7.45-7.54 (m, 2H, aromatic), 8.36 (br s, 1H, NH), 9.59 (br s, 1H, NH), 10.34 (br s, 1H, NH). Anal. Calcd for $C_{13}H_{15}N_3O_5S$: C, 47.99; H, 4.65; N, 12.92; S, 9.86. Found: C, 48.02; H, 4.55; N, 12.89; S, 9.79.

A solution of 1,3-benzodioxol-5-yl thiosemicarbazide (1.04 g, 3.2 mmol) in 20 mL of 5% NaOH was stirred under reflux for 30 min. After cooling, the solution was acidified by the addition of 5% HCl. The resulting solid was filtered off, washed with water and recrystallized from EtOH to give 0.67 g (75%) of **4c** as a white powder: mp 247-249 °C. $R_f$ = 0.35 (ethyl acetate/methanol 7:3 v/v). $^1$H NMR (DMSO-$d_6$) δ 4.79 (s, 2H, $NCH_2$), 6.13 (s, 2H, $OCH_2O$), 7.11-7.15 (m, 3H, aromatic), 13.41 (br s, 1H, COOH), 14.01 (br s, 1H, SH). Anal. Calcd for $C_{11}H_9N_3O_4S$: C, 47.31; H, 3.25; N, 15.05; S, 11.48. Found: C, 47.55; H, 3.32; N, 14.99; S, 11.39.

**General Procedure for the Preparation of [1,2,4]triazole Derivatives 5a-n**

A mixture of the appropriate triazole **4a-g** (2.1 mmol) and $K_2CO_3$ (3.1 mmol), in 15 mL of acetone and arylmethyl-chloride or ethyl chloroacetate (2.1 mmol), was stirred at room temperature for 1 h. After concentration of the solvent, ice was added and the medium was acidified with 2N HCl. The obtained suspension was filtered and solids were washed with water and recrystallized from EtOH to give **5a-g** or **5i-m** as white powders (yields 50-70%). In the case of ester **5h**, the crude material obtained after acidification, was extracted with $CH_2Cl_2$ (3 × 50 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered and concentrated under reduced pressure; the obtained crude residue was used without further purification.

An ethanolic solution of ester **5h** (2.0 mmol) and 5% NaOH (3.5 mmol) was refluxed for 30 min under stirring. After cooling, the solution was acidified by the addition of 5% HCl. The resulting solid was filtered off, washed with water and recrystallized from ethanol to give **5n** as white powder with a yield of 80%. Using these procedures the following compounds were obtained.

***[3-[[(4-Methoxyphenyl)methyl]thio]-5-phenyl-4H-[1,2,4]triazol-4-yl]acetic Acid 5a***

Recrystallization from absolute ethanol gave 0.49 g (65%) of **5a** as a white solid: mp 160-162 °C. $R_f$ = 0.38 (ethyl acetate/methanol 7:3 v/v). IR (KBr, selected lines) cm$^{-1}$ 3100, 3004, 2833, 1722, 1610, 1513, 1303, 1218, 1028, 694. $^1$H NMR (DMSO-$d_6$) δ 3.73 (s, 3H, $OCH_3$), 4.31 (s, 2H, $SCH_2$), 4.64 (s, 2H, $NCH_2$), 6.86-6.90 (m, 2H, aromatic), 7.25-7.29 (m, 2H, aromatic), 7.51-7.59 (m, 5H, aromatic), 13.61 (br s, 1H, COOH). Anal. Calcd for $C_{18}H_{17}N_3O_3S$: C, 60.83; H, 4.82; N, 11.82; S, 9.02. Found: C, 60.54; H, 4.79; N, 11.91; S, 9.12.

***[3-[[(4-Methoxyphenyl)methyl]thio]-5-(4-methoxyphenyl)-4H-[1,2,4]triazol-4-yl]acetic Acid 5b***

Recrystallization from absolute ethanol gave 0.56 g (70%) of **5b** as a white solid: mp 163-165 °C. $R_f$ = 0.45 (ethyl acetate/methanol 7:3 v/v). IR (KBr, selected lines) cm$^{-1}$ 3130, 3005, 2932, 2832, 1724, 1611, 1512, 1461, 1252, 1179, 1028, 841. $^1$H NMR (DMSO-$d_6$) δ 3.73 (s, 3H, $OCH_3$), 3.82 (s, 3H, $OCH_3$), 4.29 (s, 2H, $SCH_2$), 4.60 (s, 2H, $NCH_2$), 6.82-6.92 (m, 2H, aromatic), 7.08-7.12 (m, 2H, aromatic), 7.24-7.28 (m, 2H, aromatic), 7.45-7.49 (m, 2H, aromatic). Anal. Calcd for $C_{19}H_{19}N_3O_4S$: C, 59.21; H, 4.97; N, 10.90; S, 8.32. Found: C, 59.39; H, 4.88; N, 11.11; S, 8.29.

***[3-[[(1,3-Benzodioxol-5-yl)methyl]thio]-5-(4-methoxyphenyl)-4H-[1,2,4]triazol-4-yl]acetic Acid 5c***

Recrystallization from absolute ethanol gave 0.50 g (60%) of **5c** as a white solid: mp 168-170 °C. $R_f$ = 0.55 (ethyl acetate/methanol 7.3 v/v). IR (KBr, selected lines) cm$^{-1}$ 3100, 3002, 2908, 2839, 1728, 1612, 1490, 1444, 1360, 1181, 1026, 841. $^1$H NMR (DMSO-$d_6$) δ 3.82 (s, 3H, $OCH_3$), 4.26 (s, 2H, $SCH_2$), 4.64 (s, 2H, $NCH_2$), 6.00 (s, 2H, $OCH_2O$), 6.81-6.83 (m, 2H, aromatic), 6.93-6.94 (m, 1H, aromatic), 7.08-7.12 (m, 2H, aromatic), 7.46-7.50 (m, 2H, aromatic). Anal. Calcd for $C_{19}H_{17}N_3O_5S$: C, 57.13; H, 4.29; N, 10.52; S, 8.03. Found: C, 57.29; H, 4.32; N, 10.63; S, 7.99.

***[5-(1,3-Benzodioxol-5-yl)-3-[[(4-methoxyphenyl)methyl]thio]-4H-[1,2,4]triazol-4-yl]acetic Acid 5d***

Recrystallization from absolute ethanol gave 0.53 g (65%) of **5d** as a white solid: mp 158-160 °C. $R_f$ = 0.43 (ethyl acetate/methanol 7:3 v/v). IR (KBr, selected lines) cm$^{-1}$ 3425, 3010, 2894, 2836, 1722, 1608, 1511, 1469, 1231, 1035, 828. $^1$H NMR (DMSO-$d_6$) δ 3.73 (s, 3H, $OCH_3$), 4.29 (s, 2H, $SCH_2$), 4.62 (s, 2H, $NCH_2$), 6.13 (s, 2H, $OCH_2O$), 6.86-6.90 (m, 2H, aromatic), 6.98-7.11 (m, 3H, aromatic), 7.24-7.29 (m, 2H, aromatic). Anal. Calcd for $C_{19}H_{17}N_3O_5S$: C, 57.13; H, 4.29; N, 10.52; S, 8.03. Found: C, 57.32; H, 4.22; N, 10.61; S, 8.15.

***[5-(1,3-Benzodioxol-5-yl)-3-[[(2-methoxyphenyl)methyl]thio]-4H-[1,2,4]triazol-4-yl]acetic Acid 5e***

Recrystallization from absolute ethanol gave 0.45 g (55%) of **5e** as a white solid: mp 175-178 °C. $R_f$ = 0.52 (ethyl acetate/methanol 7:3 v/v). IR (KBr, selected lines) cm$^{-1}$ 3100, 3063, 2942, 2895, 1724, 1596, 1461, 1341, 1253, 1042, 878. $^1$H NMR (DMSO-$d_6$) δ 3.79 (s, 3H, $OCH_3$), 4.27 (s, 2H, $SCH_2$), 4.64 (s, 2H, $NCH_2$), 6.13 (s, 2H, $OCH_2O$), 6.84-6.92 (m, 2H, aromatic), 6.99-7.11 (m, 3H, aromatic), 7.22-7.33 (m, 2H, aromatic), 13.05 (br s, 1H, COOH). Anal. Calcd for $C_{19}H_{17}N_3O_5S$: C, 57.13; H, 4.29; N, 10.52; S, 8.03. Found: C, 56.99; H, 4.33; N, 10.62; S, 8.12.

***[5-(1,3-Benzodioxol-5-yl)-3-[[(3-methoxyphenyl)methyl]thio]-4H-[1,2,4]triazol-4-yl]acetic Acid 5f***

Recrystallization from absolute ethanol gave 0.41 g (50%) of **5f** as a white solid: mp 145-148 °C. $R_f$ = 0.41 (ethyl acetate/methanol 7:3 v/v). IR (KBr, selected lines) cm$^{-1}$ 3400, 3100, 2988, 1722, 1598, 1469, 1228, 1037, 933, 824. $^1$H NMR (DMSO-$d_6$) δ 3.70 (s, 3H, $OCH_3$), 4.29 (s, 2H, $SCH_2$), 4.59 (s, 2H, $NCH_2$), 6.12 (s, 2H, $OCH_2O$), 6.82-7.11 (m, 6H, aromatic), 7.19-7.27 (m, 1H, aromatic). Anal. Calcd for $C_{19}H_{17}N_3O_5S$: C, 57.13; H, 4.29; N, 10.52; S, 8.03. Found: C, 57.28; H, 4.31; N, 10.44; S, 8.29.

***[5-(1,3-Benzodioxol-5-yl)-3-[[(4-methylthiophenyl)methyl]thio]-4H-[1,2,4]triazol-4-yl]acetic Acid 5g***

Recrystallization from absolute ethanol gave 0.43 g (50%) of **5g** as a white solid: mp 155-157 °C. $R_f$ = 0.52 (ethyl acetate/methanol 7:3 v/v). IR (KBr, selected lines) cm$^{-1}$ 3100, 2984, 1722, 1605, 1474, 1428, 1240, 1088, 1034, 932, 825. $^1$H NMR (DMSO-$d_6$) δ 2.44 (s, 3H, $SCH_3$), 4.30 (s, 2H, $SCH_2$), 4.63 (s, 2H, $NCH_2$), 6.12 (s, 2H, $OCH_2O$), 6.97-7.10

(m, 3H, aromatic), 7.17-7.30 (m, 4H, aromatic). Anal. Calcd for $C_{19}H_{17}N_3O_4S_2$: C, 54.93; H, 4.12; N, 10.11; S, 15.43. Found: C, 55.12; H, 4.23; N, 10.22; S, 15.29.

***[[5-Phenyl-4-(phenylmethyl)-4H-[1,2,4]-triazol-3-yl]thio]acetic Acid Ethyl Ester 5h***

Analytical data are in agreement with those reported in reference 24.

***3-[[(4-Methoxyphenyl)methyl]thio]-5-phenyl-1H-[1,2,4]triazole 5i***

Recrystallization from absolute ethanol gave 0.43 g (70%) of **5i** as a white solid: mp 140-142 °C. $R_f$ = 0.53 (ethyl acetate/cyclohexane 3:7 v/v). $^1$H NMR (DMSO-$d_6$) δ 3.71 (s, 3H, $OCH_3$), 4.39 (s, 2H, $SCH_2$), 6.85-6.89 (m, 2H, aromatic), 7.33-7.37 (m, 2H, aromatic), 7.49-7.52 (m, 3H, aromatic), 8.01-8.02 (m, 2H, aromatic), 14.25 (br s, 1H, NH). Anal. Calcd for $C_{16}H_{15}N_3OS$: C, 64.62; H, 5.08; N, 14.13; S, 10.78. Found: C, 64.81; H, 5.19; N, 13.98; S, 10.54.

***3-[[(4-Methoxyphenyl)methyl]thio]-5-(4-methoxyphenyl)-1H-[1,2,4]triazole 5j***

Analytical data are in agreement with those reported in reference 25.

***3-[[(1,3-Benzodioxol-5-yl)methyl]thio]-5-(4-methoxyphenyl)-1H-[1,2,4]triazole 5k***

Recrystallization from absolute ethanol gave 0.61 g (65%) of **5k** as a white solid: mp 147-149 °C. $R_f$ = 0.43 (ethyl acetate/cyclohexane 3:7 v/v). $^1$H NMR(DMSO-$d_6$) δ 3.81 (s, 3H, $OCH_3$), 4.31 (s, 2H, $SCH_2$), 5.98 (s, 2H, $OCH_2O$), 6.84-6.86 (m, 2H, aromatic), 6.91-7.09 (m, 3H, aromatic), 7.88-7.93 (m, 2H, aromatic), 14.28 (br s, 1H, NH). Anal. Calcd for $C_{17}H_{15}N_3O_3S$: C, 59.81; H, 4.43; N, 12.31; S, 9.39. Found: C, 60.01; H, 4.52; N, 12.12; S, 9.11.

***5-(1,3-Benzodioxol-5-yl)-3-[[(4-methoxyphenyl)methyl]thio]-1H-[1,2,4]triazole 5l***

Recrystallization from absolute ethanol gave 0.63 g (68%) of **5l** as a white solid: mp 149-151 °C. $R_f$ = 0.34 (ethyl acetate/cyclohexane 3:7 v/v). $^1$H NMR (DMSO-$d_6$) δ 3.71 (s, 3H, $OCH_3$), 4.35 (s, 2H, $SCH_2$), 6.13 (s, 2H, $OCH_2O$), 6.86-6.90 (m, 2H, aromatic), 6.98-7.11 (m, 3H, aromatic), 7.24-7.29 (m, 2H, aromatic). Anal. Calcd for $C_{17}H_{15}N_3O_3S$: C, 59.81; H, 4.43; N, 12.31; S, 9.39. Found: C, 59.73; H, 4.55; N, 12.12; S, 9.52.

***[(5-Phenyl-1H-[1,2,4]triazol-3-yl)thio]acetic Acid Ethyl Ester 5m***

Analytical data are in agreement with those reported in reference 26.

***[[5-Phenyl-4-(phenylmethyl)-4H-[1,2,4]triazol-3-yl]thio]acetic Acid 5n***

Recrystallization from absolute ethanol gave 0.52 g (80%) of **5n** as a white solid: mp 174-176 °C. $R_f$ = 0.34 (ethyl acetate/methanol 7:3 v/v). IR (KBr, selected lines) $cm^{-1}$ 3099, 3055, 2926, 1720, 1610, 1490, 1370, 1325, 1190, 1018, 731. $^1$H NMR (DMSO-$d_6$) δ 4.07 (s, 2H, $SCH_2$), 5.28 (s, 2H, $NCH_2$), 6.95-6.99 (m, 2H, aromatic), 7.29-7.32 (m, 3H, aromatic), 7.48-7.57 (m, 5H, aromatic), 13.01 (br s, 1H, COOH). Anal. Calcd for $C_{17}H_{15}N_3O_2S$: C, 62.75; H, 4.65; N, 12.91; S, 9.85. Found: C, 62.93; H, 4.54; N, 12.77; S, 9.69.

**General Procedure for the Preparation of [1,2,4]triazole Derivatives 8a-e and 9a-e**

To a mixture of the appropriate triazole **5i-m** (3.0 mmol), $K_2CO_3$ (4.5 mmol), and a catalytic amount of KI in 25 mL of acetone, ethyl chloroacetate or benzyl chloride (4.5 mmol) was added dropwise. The mixture was stirred under reflux for 5-6 h; after this time, the solvent was removed *in vacuo*. The obtained crude material was diluted with water and extracted with $CH_2Cl_2$ (3 × 50 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered and concentrated under reduced pressure. The oily residues, which contained both regioisomers **6a-e** and **7a-e**, were purified by flash chromatography performed using silica gel 60 (230-400 mesh) and a mixture of ethyl acetate/cyclohexane 3:7 v/v as eluent. The first collected homogeneous fractions were evaporated *in vacuo* to give separated esters of the series **6** as oils; subsequent elution gave esters of the series **7** as oils.

A solution of the appropriate ester **6a-e** or **7a-e** (2.0 mmol) and 5% NaOH (3.5 mmol) in 10-15 mL of EtOH was refluxed for 30 min under stirring. After cooling, the mixture was acidified by the addition of 5% HCl. The resulting suspensions were filtered, and obtained solids were washed with water and recrystallized from ethanol to give final acids **8a-e** and **9a-e** as white powders in 70-80% yields. Using this procedure the following compounds were obtained.

***[5-[[(4-Methoxyphenyl)methyl]thio]-3-phenyl-1H-[1,2,4]triazol-1-yl]acetic Acid Ethyl Ester 6a***

Flash chromatography purification gave 52% of **6a** as a thick colorless oil. $R_f$ = 0.48 (ethyl acetate/cyclohexane 3:7 v/v).$^1$H NMR (DMSO-$d_6$) δ 1.19 (t, J = 7.2 Hz, 3H, $CH_2CH_3$), 3.71 (s, 3H, $OCH_3$), 4.17 (q, J = 7.2 Hz, 2H, $CH_2CH_3$), 4.44 (s, 2H, $NCH_2$), 5.03 (s, 2H, $NCH_2$), 6.85-6.89 (m, 2H, aromatic), 7.32-7.35 (m, 2H, aromatic), 7.46-7.49 (m, 3H, aromatic), 7.98-8.03 (m, 2H, aromatic). Anal. Calcd for $C_{20}H_{21}N_3O_3S$: C, 62.64; H, 5.52; N, 10.96; S, 8.36. Found: C, 62.47; H, 5.68; N, 10.79; S, 8.21.

***[5-[[(4-Methoxyphenyl)methyl]thio]-3-(4-methoxyphenyl)-1H-[1,2,4]triazol-1-yl]acetic Acid Ethyl Ester 6b***

Flash chromatography purification gave 50% of **6b** as a thick colorless oil. $R_f$ = 0.45 (ethyl acetate/cyclohexane 3:7 v/v). $^1$H NMR (DMSO-$d_6$) δ 1.18 (t, J = 7.2 Hz, 3H, $CH_2CH_3$), 3.71 (s, 3H, $OCH_3$), 3.81 (s, 3H, $OCH_3$), 4.13 (q, J = 7.2 Hz, 2H, $CH_2CH_3$), 4.41 (s, 2H, $SCH_2$), 5.01 (s, 2H, $NCH_2$), 6.84-6.88 (m, 2H, aromatic), 7.02-7.08 (m, 2H, aromatic), 7.33-7.36 (m, 2H, aromatic), 7.91-7.96 (m, 2H, aromatic). Anal. Calcd for $C_{21}H_{23}N_3O_4S$: C, 61.00; H, 5.61; N, 10.16; S, 7.75. Found: C, 61.12; H, 5.77; N, 10.22; S, 7.66.

***[5-[[(1,3-Benzodioxol-5-yl)methyl]thio]-3-(4-methoxyphenyl)-1H-[1,2,4]triazol-1-yl]acetic Acid Ethyl Ester 6c***

Flash chromatography purification gave 43% of **6c** as a thick colorless oil. $R_f$ = 0.44 (ethyl acetate/cyclohexane 3:7 v/v). $^1$H NMR (DMSO-$d_6$) δ 1.19 (t, J = 7.2 Hz, 3H, $CH_2CH_3$), 3.81 (s, 3H, $OCH_3$), 4.14 (q, J = 7.2 Hz, 2H, $CH_2CH_3$), 4.41 (s, 2H, $SCH_2$), 5.02 (s, 2H, $NCH_2$), 5.98 (s, 2H, $OCH_2O$), 6.84-7.06 (m, 5H, aromatic), 7.90-7.94 (m,

2H, aromatic). Anal. Calcd for $C_{21}H_{21}N_3O_5S$: C, 59.00; H, 4.95; N, 9.83; S, 7.50. Found: C, 59.28; H, 4.86; N, 9.79; S, 7.43.

### *[3-(1,3-Benzodioxol-5-yl)-5-[[(4-methoxyphenyl)methyl] thio]-1H-[1,2,4]triazol-1-yl]acetic Acid Ethyl Ester 6d*

Flash chromatography purification gave 40% of **6d** as a thick colorless oil. $R_f$ = 0.45 (ethyl acetate/cyclohexane 3:7 v/v). $^1$H NMR (DMSO-$d_6$) δ 1.18 (t, J = 7.2 Hz, 3H, $CH_2CH_3$), 3.71 (s, 3H, $OCH_3$), 4.13 (q, J = 7.2 Hz, 2H, $CH_2CH_3$), 4.42 (s, 2H, $SCH_2$), 4.99 (s, 2H, $NCH_2$), 6.09 (s, 2H, $OCH_2O$), 6.84-6.88 (m, 2H, aromatic), 7.00-7.04 (m, 1H, aromatic), 7.30-7.34 (m, 2H, aromatic), 7.42-7.57 (m, 2H, aromatic). Anal. Calcd for $C_{21}H_{21}N_3O_5S$: C, 59.00; H, 4.95; N, 9.83; S, 7.50. Found: C, 59.21; H, 4.82; N, 9.72; S, 7.68.

### *[[1-(Phenylmethyl)-3-phenyl-1H-[1,2,4]triazol-5-yl]thio]acetic Acid Ethyl Ester 6e*

Flash chromatography purification gave 35% of **6e** as a thick colorless oil. $R_f$ = 0.48 (ethyl acetate/cyclohexane 3:7 v/v). $^1$H NMR (DMSO-$d_6$) δ 1.16 (t, J = 7.0 Hz, 3H, $CH_2CH_3$), 4.14 (q, J = 7.0 Hz, 2H, $CH_2CH_3$), 4.19 (s, 2H, $SCH_2$), 5.39 (s, 2H, $NCH_2$), 7.27-7.48 (m, 8H, aromatic), 7.92-7.97 (m, 2H, aromatic). Anal. Calcd for $C_{19}H_{19}N_3O_2S$: C, 64.57; H, 5.42; N, 11.89; S, 9.07. Found: C, 64.68; H, 5.38; N, 11.71; S, 9.15.

### *[3-[[(4-Methoxyphenyl)methyl]thio]-5-phenyl-1H-[1,2,4] triazol-1-yl]acetic Acid Ethyl Ester 7a*

Flash chromatography purification gave 28% of **7a** as a thick colorless oil. $R_f$ = 0.24 (ethyl acetate/cyclohexane 3:7 v/v). $^1$H NMR (DMSO-$d_6$) δ 1.13 (t, J = 7.2 Hz, 3H, $CH_2CH_3$), 3.72 (s, 3H, $OCH_3$), 4.14 (q, J = 7.2 Hz, 2H, $CH_2CH_3$), 4.32 (s, 2H, $SCH_2$), 5.18 (s, 2H, $NCH_2$), 6.84-6.88 (m, 2H, aromatic), 7.32-7.36 (m, 2H, aromatic), 7.44-7.55 (m, 3H, aromatic), 7.67-7.7 (m, 2H, aromatic). Anal. Calcd for $C_{20}H_{21}N_3O_3S$: C, 62.64; H, 5.52; N, 10.96; S, 8.36. Found: C, 62.78; H, 5.65; N, 10.81; S, 8.23.

### *[3-[[(4-Methoxyphenyl)methyl]thio]-5-(4-methoxyphenyl)-1H-[1,2,4]triazol-1-yl]acetic Acid Ethyl Ester 7b*

Flash chromatography purification gave 20% of **7b** as a thick colorless oil. $R_f$ = 0.29 (ethyl acetate/cyclohexane 3:7 v/v). $^1$H NMR (DMSO-$d_6$) δ 1.14 (t, J = 7.2 Hz, 3H, $CH_2CH_3$), 3.71 (s, 3H, $OCH_3$), 3.81 (s, 3H, $OCH_3$), 4.13 (q, J = 7.2 Hz, 2H, $CH_2CH_3$), 4.31 (s, 2H, $SCH_2$), 5.14 (s, 2H, $NCH_2$), 6.84-6.88 (m, 2H, aromatic), 7.08-7.13 (m, 2H, aromatic), 7.32-7.36 (m, 2H, aromatic), 7.59-7.63 (m, 2H, aromatic). Anal. Calcd for $C_{21}H_{23}N_3O_4S$: C, 61.00; H, 5.61; N, 10.16; S, 7.75. Found: C, 61.15; H, 5.73; N, 10.28; S, 7.59.

### *[3-[[(1,3-Benzodioxol-5-yl)methyl]thio]-5-(4-methoxyphenyl)-1H-[1,2,4]triazol-1-yl]acetic Acid Ethyl Ester 7c*

Flash chromatography purification gave 20% of **7c** as a thick colorless oil. $R_f$ = 0.31 (ethyl acetate/cyclohexane 3:7 v/v). $^1$H NMR (DMSO-$d_6$) δ 1.17 (t, J = 7.2 Hz, 3H, $CH_2CH_3$), 3.83 (s, 3H, $OCH_3$), 4.12 (q, J = 7.2 Hz, 2H, $CH_2CH_3$), 4.29 (s, 2H, $SCH_2$), 5.15 (s, 2H, $NCH_2$), 5.98 (s, 2H, $OCH_2O$), 6.81-6.98 (m, 3H, aromatic), 7.07-7.12 (m, 2H, aromatic), 7.59-7.63 (m, 2H, aromatic). Anal. Calcd for $C_{21}H_{21}N_3O_5S$: C, 59.00; H, 4.95; N, 9.83; S, 7.50. Found: C, 59.18; H, 4.87; N, 9.75; S, 7.71.

### *[5-(1,3-Benzodioxol-5-yl)-3-[[(4-methoxyphenyl)methyl] thio]-1H-[1,2,4]triazol-1-yl]acetic Acid Ethyl Ester 7d*

Flash chromatography purification gave 25% of **7d** as a thick colorless oil. $R_f$ = 0.32 (ethyl acetate/cyclohexane 3:7 v/v). $^1$H NMR (DMSO-$d_6$) δ 1.18 (t, J = 7.2 Hz, 3H, $CH_2CH_3$), 3.72 (s, 3H, $OCH_3$), 4.13 (q, J = 7.2 Hz, 2H, $CH_2CH_3$), 4.31 (s, 2H, $SCH_2$), 5.16 (s, 2H, $NCH_2$), 6.13 (s, 2H, $OCH_2O$), 6.84-6.98 (m, 2H, aromatic), 7.00-7.19 (m, 3H, aromatic), 7.31-7.35 (m, 2H, aromatic). Anal. Calcd for $C_{21}H_{21}N_3O_5S$: C, 59.00; H, 4.95; N, 9.83; S, 7.50. Found: C, 59.22; H, 4.83; N, 9.77; S, 7.34.

### *[[1-(Phenylmethyl)-5-phenyl-1H-[1,2,4]triazol-3-yl]thio]acetic Acid Ethyl Ester 7e*

Flash chromatography purification gave 28% of **7e** as a thick colorless oil. $R_f$ = 0.29 (ethyl acetate/cyclohexane 3:7 v/v). $^1$H NMR (DMSO-$d_6$) δ 1.13 (t, J = 7.2 Hz, 3H, $CH_2CH_3$), 4.02 (s, 2H, $SCH_2$), 4.11 (q, J = 7.2 Hz, 2H, $CH_2CH_3$), 5.44 (s, 2H, $NCH_2$), 7.07-7.12 (m, 2H, aromatic), 7.28-7.38 (m, 3H, aromatic), 7.51-7.65 (m, 5H, aromatic). Anal. Calcd for $C_{19}H_{19}N_3O_2S$: C, 64.57; H, 5.42; N, 11.89; S, 9.07. Found: C, 64.39; H, 5.49; N, 11.77; S, 9.19.

### *[5-[[(4-Methoxyphenyl)methyl]thio]-3-phenyl-1H-[1,2,4] triazol-1-yl]acetic Acid 8a*

Recrystallization from absolute ethanol gave 0.56 g (80%) of **8a** as a white solid: mp 160-162 °C. $R_f$ = 0.5 (ethyl acetate/methanol 7:3 v/v). IR (KBr, selected lines) cm$^{-1}$ 3100, 2997, 2946, 2839, 1715, 1611, 1515, 1440, 1403, 1228, 1021, 726. $^1$H NMR (DMSO-$d_6$) δ 3.71 (s, 3H, $OCH_3$), 4.43 (s, 2H, $SCH_2$), 4.92 (s, 2H, $NCH_2$), 6.84-6.89 (m, 2H, aromatic), 7.31-7.36 (m, 2H, aromatic), 7.46-7.52 (m, 3H, aromatic), 7.98-8.02 (m, 2H, aromatic), 14.07 (br s, 1H, COOH). Anal. Calcd for $C_{18}H_{17}N_3O_3S$: C, 60.83; H, 4.82; N, 11.82; S, 9.02. Found: C, 60.67; H, 4.92; N, 11.69; S, 9.18.

### *[5-[[(4-Methoxyphenyl)methyl]thio]-3-(4-methoxyphenyl)-1H-[1,2,4]triazol-1-yl]acetic Acid 8b*

Recrystallization from absolute ethanol gave 0.55 g (72%) of **8b** as a white solid: mp 158-160 °C. $R_f$ = 0.46 (ethyl acetate/methanol 7:3 v/v). IR (KBr, selected lines) cm$^{-1}$ 3425, 2900, 1724, 1610, 1512, 1464, 1303, 1241, 1178, 1036, 821. $^1$H NMR (DMSO-$d_6$) δ 3.71 (s, 3H, $OCH_3$), 3.81 (s, 3H, $OCH_3$), 4.41 (s, 2H, $SCH_2$), 4.87 (s, 2H, $NCH_2$), 6.84-6.88 (m, 2H, aromatic), 7.01-7.06 (m, 2H, aromatic), 7.31-7.35 (m, 2H, aromatic), 7.90-7.95 (m, 2H, aromatic), 13.56 (br s, 1H, COOH). Anal. Calcd for $C_{19}H_{19}N_3O_4S$: C, 59.21; H, 4.97; N, 10.90; S, 8.32. Found: C, 59.43; H, 4.83; N, 10.75; S, 8.41.

### *[5-[[(1,3-Benzodioxol-5-yl)methyl]thio]-3-(4-methoxyphenyl)-1H-[1,2,4]triazol-1-yl]acetic Acid 8c*

Recrystallization from absolute ethanol gave 0.64 g (80%) of **8c** as a white solid: mp 150-152 °C. $R_f$ = 0.55 (ethyl acetate/methanol 7:3 v/v). IR (KBr, selected lines) cm$^{-1}$ 3200, 2937, 2898, 1731, 1612, 1582, 1487, 1442, 1328, 1253, 1178, 837. $^1$H NMR (DMSO-$d_6$) δ 3.81 (s, 3H, $OCH_3$), 4.40 (s, 2H, $SCH_2$), 4.89 (s, 2H, $NCH_2$), 5.98 (s, 2H,

$OCH_2O$), 6.85-7.01 (m, 5H, aromatic), 7.90-7.93 (m, 2H, aromatic), 13.80 (br s, 1 H, COOH). Anal. Calcd for $C_{19}H_{17}N_3O_5S$: C, 57.13; H, 4.29; N, 10.52; S, 8.03. Found: C, 57.31; H, 4.18; N, 10.33; S, 8.22.

***[3-(1,3-Benzodioxol-5-yl)-5-[[(4-methoxyphenyl)methyl] thio]-1H-[1,2,4]triazol-1-yl]acetic Acid 8d***

Recrystallization from absolute ethanol gave 0.56 g (70%) of **8d** as a white solid: mp 127-129 °C. $R_f$ = 0.49 (ethyl acetate/methanol 7:3 v/v). IR (KBr, selected lines) $cm^{-1}$ 3120, 3001, 2934, 2835, 1716, 1611, 1511, 1427, 1247, 1175, 1026, 837. $^1$H NMR (DMSO-$d_6$) δ 3.71 (s, 3H, $OCH_3$), 4.40 (s, 2H, $SCH_2$), 4.85 (s, 2H, $NCH_2$), 6.08 (s, 2H, $OCH_2O$), 6.83-6.88 (m, 2H, aromatic), 6.99-7.03 (m, 1H, aromatic), 7.29-7.42 (m, 2H, aromatic), 7.51-7.56 (m, 2H, aromatic). Anal. Calcd for $C_{19}H_{17}N_3O_5S$: C, 57.13; H, 4.29; N, 10.52; S, 8.03. Found: C, 57.39; H, 4.21; N, 10.37; S, 8.27.

***[[1-(Phenylmethyl)-3-phenyl-1H-[1,2,4]triazol-5-yl]thio]acetic Acid 8e***

Recrystallization from absolute ethanol gave 0.48 g (74%) of **8e** as a white solid: mp 153-155 °C. $R_f$ = 0.48 (ethyl acetate/methanol 7:3 v/v). IR (KBr, selected lines) $cm^{-1}$ 3130, 3080, 2950, 2830, 1730, 1610, 1530, 1480, 1420, 1300, 1250, 1020, 768. $^1$H NMR (DMSO-$d_6$) δ 4.15 (s, 2H, $SCH_2$), 5.38 (s, 2H, $NCH_2$), 7.32-7.46 (m, 8H, aromatic), 7.92-7.97 (m, 2H, aromatic), 13.02 (br s, 1H, COOH). Anal. Calcd for $C_{17}H_{15}N_3O_2S$: C, 62.75; H, 4.65; N, 12.91; S, 9.85. Found: C, 62.59; H, 4.53; N, 12.79; S, 10.01.

***[3-[[(4-Methoxyphenyl)methyl]thio]-5-phenyl-1H-[1,2,4] triazol-1-yl]acetic Acid 9a***

Recrystallization from absolute ethanol gave 0.53 g (75%) of **9a** as a white solid: mp 159-160 °C. $R_f$ = 0.32 (ethyl acetate/methanol 7:3). IR (KBr, selected lines) $cm^{-1}$ 3100, 3039, 2999, 2833, 1720, 1610, 1512, 1474, 1300, 1219, 1017, 824. $^1$H NMR (DMSO-$d_6$) δ 3.72 (s, 3H, $OCH_3$), 4.32 (s, 2H, $SCH_2$), 5.06 (s, 2H, $NCH_2$), 6.84-6.89 (m, 2H, aromatic), 7.32-7.37 (m, 2H, aromatic), 7.57-7.59 (m, 3H, aromatic), 7.65-7.70 (m, 2H, aromatic). Anal. Calcd for $C_{18}H_{17}N_3O_3S$: C, 60.83; H, 4.82; N, 11.82; S, 9.02. Found: C, 60.71; H, 4.75; N, 11.69; S, 9.25.

***[3-[[(4-Methoxyphenyl)methyl]thio]-5-(4-methoxyphenyl)-1H-[1,2,4]triazol-1-yl]acetic Acid 9b***

Recrystallization from absolute ethanol gave 0.54 g (76%) of **9b** as a white solid: mp 155-157 °C. $R_f$ = 0.29 (ethyl acetate/methanol 7:3 v/v). IR (KBr, selected lines) $cm^{-1}$ 3120, 3016, 2939, 2837, 1722, 1610, 1512, 1462, 1293, 1259, 1017, 840. $^1$H NMR (DMSO-$d_6$) δ 3.72 (s, 3H, $OCH_3$), 3.82 (s, 3H, $OCH_3$), 4.31 (s, 2H, $SCH_2$), 5.03 (s, 2H, $NCH_2$), 6.84-6.88 (m, 2H, aromatic), 7.08-7.12 (m, 2H, aromatic), 7.32-7.36 (m, 2H, aromatic), 7.59-7.63 (m, 2H, aromatic), 13.51 (br s, 1H, COOH). Anal. Calcd for $C_{19}H_{19}N_3O_4S$: C, 59.21; H, 4.97; N, 10.90; S, 8.32. Found: C, 59.44; H, 4.88; N, 10.79; S, 8.44.

***[3-[[(1,3-Benzodioxol-5-yl)methyl]thio]-5-(4-methoxyphenyl)-1H-[1,2,4]triazol-1-yl]acetic Acid 9c***

Recrystallization from absolute ethanol gave 0.60 g (75%) of **9c** as a white solid: mp 170-172 °C. $R_f$ = 0.52 (ethyl acetate/methanol 7:3 v/v). IR (KBr, selected lines) $cm^{-1}$ 3062, 2946, 2844, 1722, 1612, 1487, 1307, 1257, 1182, 1036, 835. $^1$H NMR (DMSO-$d_6$) δ 3.82 (s, 3H, $OCH_3$), 4.29 (s, 2H, $SCH_2$), 5.04 (s, 2H, $NCH_2$), 5.98 (s, 2H, $OCH_2O$), 6.80-7.00 (m, 3H, aromatic), 7.08-7.14 (m, 2H, aromatic), 7.59-7.65 (m, 2H, aromatic), 13.49 (br s, 1H, COOH). Anal. Calcd for $C_{19}H_{17}N_3O_5S$: C, 57.13; H, 4.29; N, 10.52; S, 8.03. Found: C, 57.28; H, 4.34; N, 10.33; S, 8.19.

***[5-(1,3-Benzodioxol-5-yl)-3-[[(4-methoxyphenyl)methyl] thio]-1H-[1,2,4]triazol-1-yl]acetic Acid 9d***

Recrystallization from absolute ethanol gave 0.62 g (78%) of **9d** as a white solid: mp 198-199 °C. $R_f$ = 0.23 (ethyl acetate/methanol 7:3 v/v). IR (KBr, selected lines) $cm^{-1}$ 3130, 3060, 2980, 1722, 1610, 1512, 1290, 1182, 1017, 824. $^1$H NMR (DMSO-$d_6$) δ 3.71 (s, 3H, $OCH_3$), 4.31 (s, 2H, $SCH_2$), 5.06 (s, 2H, $NCH_2$), 6.12 (s, 2H, $OCH_2O$), 6.85-6.89 (m, 2H, aromatic), 7.00-7.21 (m, 3H, aromatic), 7.30-7.36 (m, 2H, aromatic). Anal. Calcd for $C_{19}H_{17}N_3O_5S$: C, 57.13; H, 4.29; N, 10.52; S, 8.03. Found: C, 57.29; H, 4.33; N, 10.38; S, 8.31.

***[[1-(Phenylmethyl)-5-phenyl-1H-[1,2,4]triazol-3-yl]thio]acetic Acid 9e***

Recrystallization from absolute ethanol gave 0.50 g (77%) of **9e** as a white solid: mp 165-167 °C. $R_f$ = 0.23 (ethyl acetate/methanol 7:3 v/v). IR (KBr, selected lines) $cm^{-1}$ 3100, 3060, 2890, 1720, 1612, 1480, 1420, 1250, 1017, 720. $^1$H NMR (DMSO-$d_6$) δ 3.98 (s, 2H, $SCH_2$), 5.44 (s, 2H, $NCH_2$), 7.08-7.13 (m, 2H, aromatic), 7.28-7.34 (m, 3H, aromatic), 7.51-7.65 (m, 5H, aromatic). Anal. Calcd for $C_{17}H_{15}N_3O_2S$: C, 62.75; H, 4.65; N, 12.91; S, 9.85. Found: C, 62.89; H, 4.73; N, 12.77; S, 9.78.

***Synthesis of [[1-(phenylmethyl)-3-phenyl-1H-[1,2,4]triazol-5-yl]thio]acetic Acid 8e***

A mixture of benzyl hydrazine hydrochloride (0.41 g, 2.1 mmol) and triethylamine (0.21 g, 2.1 mmol) in 10 mL of dry toluene was added dropwise to a stirred solution of benzoyl isothiocyanate **10** (0.37 g, 2.3 mmol) in 10 mL of dry toluene. The reaction mixture was heated at 80 °C for 30 min and it was then allowed to cool to room temperature. The reaction mixture was then diluted with 20 mL of toluene and the precipitate was collected by filtration, suspended in 20 mL of water and again filtered to give intermediate **11** as a white solid. Compound **11** was treated, without further purification, with 20 mL of 1M $NaHCO_3$ and the resulting mixture was refluxed for 1 h. After cooling, the suspension was filtered and the filtrate was acidified by the careful addition of 1M HCl. The obtained precipitate was filtered and recrystallized from EtOH affording 0.43 g (70%) of 2-phenylmethyl-5-phenyl-2,4-dihydro-3*H*-[1,2,4]triazole-3-thione **12** as a white solid [22].

Ethyl chloroacetate (4.5 mmol) was added dropwise to a suspension of triazole **12** (3.0 mmol) and $K_2CO_3$ (4.5 mmol) in 25 mL of acetone. The mixture was stirred under reflux for 5-6 h; after this time, the solvent was removed *in vacuo*. The obtained crude material was diluted with water and extracted with $CH_2Cl_2$ (3 × 50 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered and concen-

trated under reduced pressure. The oily residue was [[1-(phenylmethyl)-3-phenyl-1*H*-[1,2,4]triazol-5-yl]thio]acetic acid ethyl ester **6e** and was used in the next step.

A solution of ester **6e** (2.0 mmol) and 5% NaOH (3.5 mmol) in 10-15 mL of EtOH, was refluxed for 30 min under stirring. After cooling, the solution was acidified by the addition of 5% HCl. The resulting suspension was filtered, the obtained solid was washed and recrystallized from EtOH to give [[1-(phenylmethyl)-3-phenyl-1*H*-[1,2,4]triazol-5-yl]thio] acetic acid **8e** as a white powder. Analytical data of compounds **6e** and **8e** are in agreement with those of the same compounds prepared following general procedure described above for compounds **8a-e** and **9a-e**.

### Binding Assays

Binding of compounds **5a-g**, **8a-d,** and **9a-d** was determined using human recombinant $ET_A$ or $ET_B$ receptors (CHO-K1 cell line purchased from Euroscreen). The cells were resuspended in Tris HCl, 50 mM, pH 7.5 containing 10 mM $MgCl_2$ and used at concentration of 0.08 µg/sample for $ET_A$ receptors, and 0.7 µg/sample for $ET_B$ receptors. Assay [27] was initiated by adding 25 µL of [$^{125}$I]endothelin-1 (0.2-0.4 nM; specific activity 2000 Ci/mmole, Amersham) in 0.05% BSA protease free in a final volume of 100 µl (tubes minisorp, Nunc). Non-specific binding was obtained in the presence of 1 µM endothelin-1 and 1 µM BQ-123 for $ET_A$ receptors or 1 µM endothelin-1 and 1 µM BQ-788 for $ET_B$ receptors. Compounds were dissolved in DMSO and tested at $10^{-5}$ and $10^{-7}$ M in triplicate, inhibition curves of endothelin-1, BQ-123, BQ-788 and compounds **5d** and **8a** were obtained using 5-7 different concentrations in triplicate. The incubation (30 °C, 60 min) was stopped by dilution with cold buffer (Tris HCl, 20 mM, pH 7.5 containing 10 mM $MgCl_2$) and filtration through GF/C filters presoaked in 0.1% BSA protease free. The filters were washed three times with the same buffer using a Brandel cell harvester and were counted in a gamma counter with a 90% efficiency. Inhibition curves were analyzed using the "Allfit" [28] program and the $K_i$ values were derived from the $IC_{50}$ values using the Cheng and Prusoff equation [29].

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