Allosteric Inhibition of ET-1 Binding to ET_A Receptors by Aldoxime Derivatives

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ABSTRACT

Endothelin-1 (ET-1), a potent vasoconstrictor peptide, exerts its physiological effects by binding and activating specific G protein-coupled receptors, named ET_A and ET_B. An unique property of ET-1 is its ability to bind almost irreversibly to its receptors. Aspirin and Salicylic Acid (SA) are allosteric inhibitors of ET-1 binding to ET_A receptors [(1)]. Dihalogenated derivatives of SA have been identified as 50 times more potent allosteric inhibitors than aspirin [(2)]. In this study, we replaced carboxylic acid group of salicylate by oxime moiety with disubstitution at 3,5 position and OH group at position 2 was replaced by NH2 or H, synthesized compounds were tested as inhibitors of [^{125} I]ET-1 binding to ET_A receptors in rat embryonic cardiomyocyte (H9c2 cell) membranes. Most oximes synthesized in this study show modest activity as inhibitors of [^{125} I] ET-1binding to ET_A receptors in relation to salicylic acid derivatives reported in literature [(2)].

Keywords: Allosteric, ET_A, Aldoxime.

INTRODUCTION

Endothelins are a family of 21-amino acid peptides found in four distinct isoforms, ET-1, ET-2, ET-3 and Endothelin β or mouse Vasoactive Intestinal Contract (VIC). ET-1 is the most potent vasoconstrictor discovered to date being ten-fold more potent than angiotensin II, and the duration of pressor effects is extremely long[(3)]. ET-1 is unusual among the mammalian bioactive peptides in being released from a dual secretory pathway [(4)]. Autoradiographic experiments with [^{125}I] ET-1 have demonstrated a wide distribution of endothelin receptors in different tissues [(5-7)]. Two endothelin receptor subtypes, termed ET_A and ET_B, have been identified [(8-18)]. These receptors belong to the large family of G Protein Coupled Receptors (GPCRs). Endothelin-1 is a potent vasoconstrictor peptide [(19)], plays an important role in several diseases thought to be associated with vasoconstrictions. These are coronary vasospasm [(20)], unstable angina [(21)], myocardial infarction [(22)], cardiac insufficiency [(23)], and cerebral vasospasm associated with subarachnoid haemorrhage [(24)], and many other pathological and physiological processes [(25-34)]. Many agonists and antagonists of endothelin receptors are described in literature classified according into their chemical structure and their selectivity to receptors. They are mainly classified into peptide [(35-38)], and non-peptide antagonist which, by its turn, could be classified into sulfonamides and nonsulfonamides[(3, 36, 37, 39, 40)]. Recently, allosteric modulation inhibition of endothelin receptors ET_A has been described in literature, a high percentage of clinically-used drugs generate their therapeutic effects by

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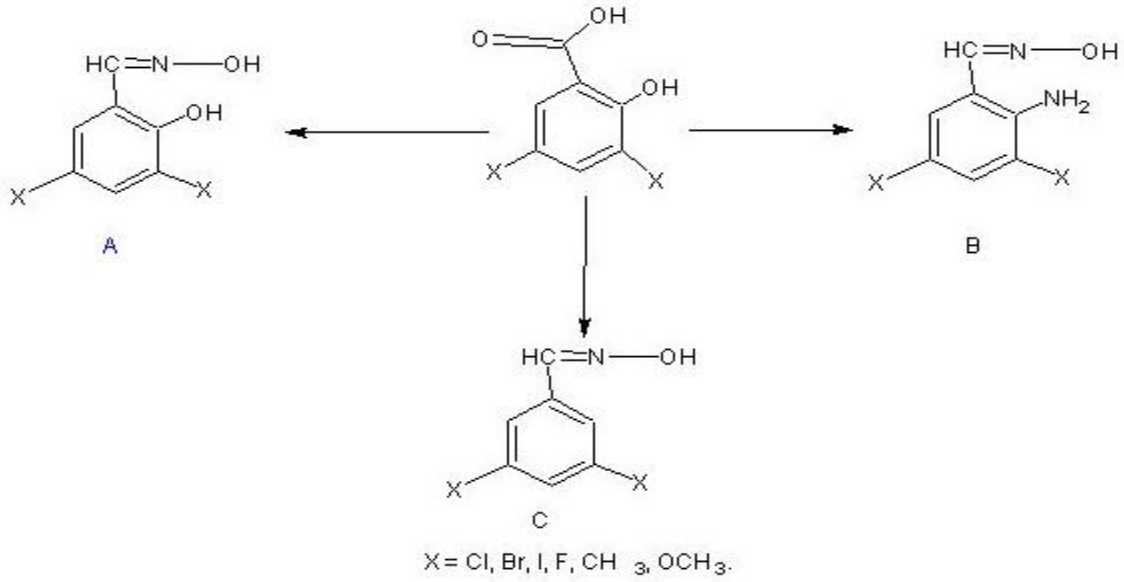
binding to G Protein-Coupled Receptors (GPCRs). Many ligands bind to GPCRs receptors type show allosteric binding type. The allosteric effect is an alternative approach in principle which is to target the drug to a second site that is different from the *Orthosteric site*, but which is conformationally linked to the Orthosteric site (i.e. *an allosteric site*). This provides the opportunity to be able to 'tune up' or 'tune down' a receptor response, rather than 'switching' it on (or off) by the use of an Orthosteric agonist or antagonist, respectively. [41] Earlier studies indicated the presence of three subtypes of endothelin receptors and possibly an allosteric interaction type, suggesting the possible existence of a specific site for BQ-123 that interacts and/or interferes with the properties of endothelin-binding sites [42]. More recent studies showed that salicylates are allosteric inhibitors of ET_A receptors [1]; this led to screen a number of derivatives of salicylic and benzoic acids to obtain compounds that would be more potent. This procedure led to the identification of dihalogenated derivatives of salicylic acid that are about 50 times more potent than aspirin. Results show that the hydroxyl group of salicylate contributes little to the effect for related molecules in the benzoic acid, and salicylic acid series are equally potent [2]. Substitution of the aromatic ring hydrogen's with halogens dramatically improves activity. Actions of halogens in the benzoic acid series follow the following rank order of potency: Br > Cl > F. All dihalogenated derivatives of salicylic acid are equipotent. 3, 5-diisopropylsalicylic acid is almost as potent as dihalogenated derivatives. These indicate that bulky groups at positions 3 and 5 of the aromatic ring of benzoic acid or of salicylic acid favour activity of the compounds [2].The aim of this research is to develop new compounds with potential effect on ET receptors level; mainly on the derivatives of dihalo-salicylic, dihalo-anthranilic and dihalo-benzoic acids which act as allosteric inhibitors. Therefore, it has synthesized the 3, 5 dihalo-substituted oximes compounds of type **A, B, C** (**scheme 1**). In these compounds, the carboxylic function of salicylic acid derivatives is substituted with an oxime moiety which possess different acid properties.

CHEMISTRY

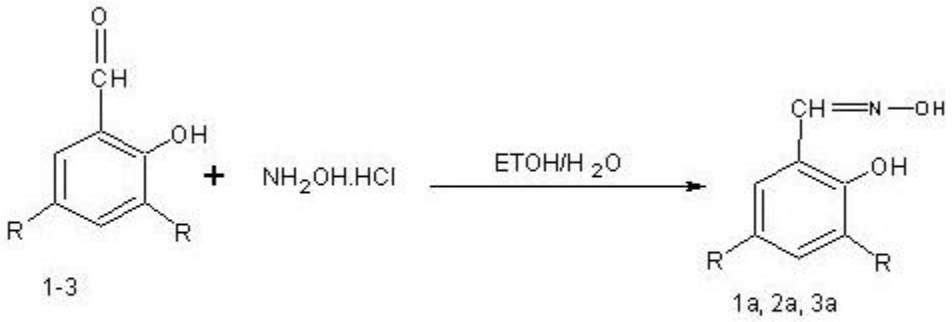
3, 5- disubstituted aryl-aldoximes compounds of type **A, B, C,** have been synthesized by condensation of an aldehyde with hydroxylamine, **Scheme 1**.

Scheme 1



3, 5-Dihalosubstituted salicyldoximes of type **A** have been synthesized as reported in **Scheme 2**. Compounds **1a, 2a** and **3a** were obtained by treating the disubstituted salicyldehyde **1-3** with hydroxylamine [43] in ethanol/water.

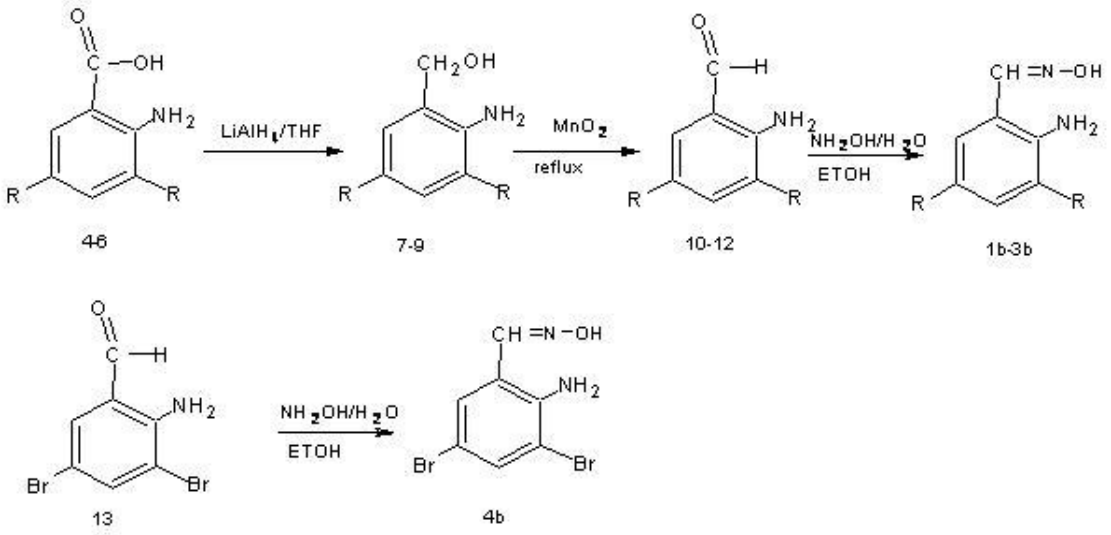
Scheme 2



1a, R = I, 2a, R= Br, 3a, R= Cl

3, 5-disubstituted-2-aminobenzoaldoximes of type **b** were prepared as shown in **Scheme 3**. The corresponding alcohols **7-9** were obtained by the reduction of 3, 5-disubstituted-2-aminobenzoic acids **4-6**, the obtained alcohols **7-9** were oxidized [4] to gave the 3, 5-disubstituted-2-amino-benzaldehydes **10-12**. The subsequent treatment of compounds **10-12** with hydroxylamine hydrochloride affords the desired compounds **1b-3b**, dibromo-aldehyde analogue **13** is available commercially, and which was treated directly with hydroxylamine hydrochloride to give **4b**.

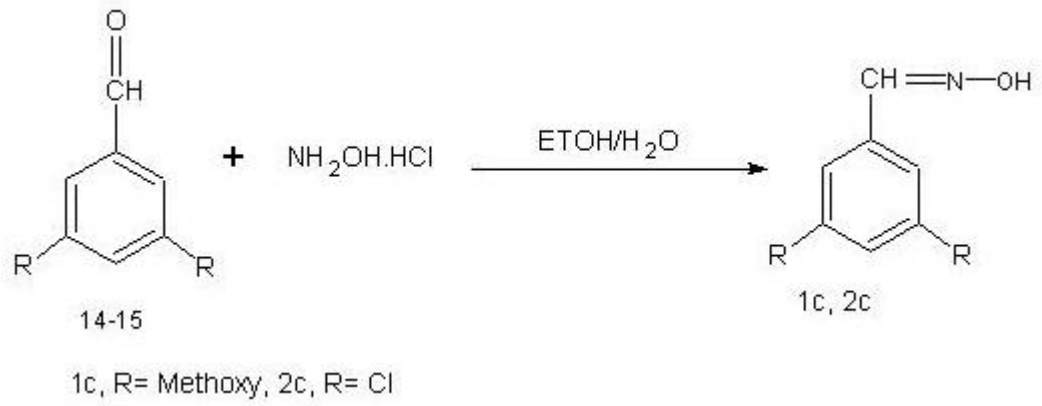
Scheme3



1b, R = CH_3; 2b, R = Cl; 3b, R = I.

The 3,5-disubstitutedbenzaldoximes compounds of type **c**, compounds 3,5-dimethoxylbenzaldoximes **1c**, and 3,5-dichlorobenzaldoximes **2c** have been synthesized according to the method described in **scheme 4**, starting with the corresponding aldehyde **14** and **15** which were treated with $NH_2OH{\cdot}HCl$ in ethanol in presence of NaOH.

Scheme 4



BIOLOGICAL STUDY

Materials and methods

Cell culture: H9c2 rat cardiomoblasts were propagated in Dulbecco's Modified Eagles's Medium (DMEM) supplemented with 10% FBS, 1 mM pyruvate, 100 units/ml penicillin, 100 μg/ml streptomycin and 0,02 mg/ml2,4-difluoro-α,α[1]-bis(1H-1,2,4-triazol-1-ylmethyl) benzyl alcohol (fluconazole), at 37° in a humidified atmosphere containing 5% CO_2 and subcultured before confluence.

Membrane preparation: Membrane preparation was performed as described by Ceccarelli *et al.*[44]. Subconfluent monolayers (passage 18-21) premium due were washed with 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.4, 136.8 mM NaCl and 2.7 mM KCl (PBS) 3 times, harvested with a cell scraper and collected by centrifugation at 1000 *g*. Cells were homogenized in 10 mM Tris-HCl, pH 7.3, containing 1 mM EDTA, 160 μg/ml benzamidine, 200 μg/ml bacitracin, 0,1 mM phenylmethanesulphonyl fluoride (PMSF) and 20 μg/ml trypsin inhibitor (buffer A) using a Polytron homogenizer. The homogenate was centrifuged at 48,000 g at 4° for 30 min. The resulting pellet was resuspended in buffer A, homogenized and centrifuged as described earlier. The membrane pellet was stored in aliquots at-80° until the time of assay. Protein concentration was determined by the Coomassie Blue binding method,[45] using bovine serum albumin (BSA) as a standard.

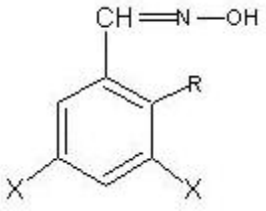
Binding assay: [^{125}I] ET-1 binding assays were performed as described by Ceccarelli et al.[79]. Cells membrane (~ 30 μg of proteins) were incubated with [^{125}I]ET-1 (~ 20 pM) in 250 ml of 20 mM Tris-HCl buffer, pH 7.4, at 37°C, containing 2 mM EDTA, 0,1 mM bacitracin, 0,1 mM PMSF, 1 μg/ml leupeptin, 5 μg/ml aprotinin (buffer B) and 0,08 mg/ml BSA for 2 hr at 37°C. After incubation, the reaction was stopped with 3 ml of ice cold 50 mM Tris-HCl, pH 7, 3 at 4°C, containing 0,1 mM bacitracin (buffer C). Membrane bound radioactivity was separated from the free ligand by filtration through Whatman GF/C filters that had been pre-soaked in buffer C containing 2 mg/ml BSA. The filters were washed three times with 3 ml of buffer C. Non-specific binding was defined as the binding that occurred in the presence of an excess of ET-1 (100 nM).

At 20 pM [^{125}I] ET-1, specific binding was about 80% of the total binding. Tested compounds were dissolved in buffer B without protease inhibitors and diluted to the desired concentration to have 10x stock solutions for the assay.

RESULTS AND DISCUSSION

All compounds, **1a-3a,1b-4b, 1c-2c**, were tested in a preliminary screening, at dose of 100 μM, to evaluate their ability to inhibit specific [^{125}I]ET-1 binding to H9c2 membranes, the results are shown in **Table (1)**, these compounds **1a-3a,1b-4b, 1c-2c** were designed as analogues of previously studied [1, 2] 3, 5-disubstitutedsalicylic acids with the aim to obtain more potent allosteric inhibitors of ET_A receptors. As shown in **Table (1)**, the benzaldoxime derivatives 1a-3a, which present in their structure hydroxyl group or hydrogen at position 2, showed modest inhibitory activity than that of 3, 5-disubstitutedsalicylic acids reported in the literature [2]. While these compounds which present NH_2 group at position 2 were completely inactive. Many competitive antagonists reported in literature are active in nM concentration while all allosteric inhibitors reported are active in mM concentration; nevertheless, even at this concentration it has a great advantage that can generate new form of selectivity beside the fact that their activity mediated only in the presence of the original ligand; which means that they devoid from many non allosteric antagonist adverse effects. Moreover, it can potentiate the competitive antagonist effects.

Tables 1: Preliminary screening of compounds synthesised (100 μM), average of three separate experiments. Values are expressed as percent of inhibition of [^{125}I] ET-1 binding to H9c2 membranes



Compound	X	R	Yield%	Melting point	M.W	*M.F*	% inhibition of [^{125}I]ET_A
1a	I	*OH*	*60.0*	*225°C*	*389*	$C_7H_5NO_2I_2$	8
2a	Br	*OH*	*86*	*207-210°C*	*293*	$C_7H_5NO_2Br_2$	16
3a	Cl	*OH*	*62*	*195-197°C*	*205*	$C_7H_5NO_2Cl_2$	10
1b	CH_3	NH_2	*60*	*180°C*	*164*	$C_9H_{16}N_2O$	0
2b	Cl	NH_2	*51.54*	*176-177°C*	*205*	$C_7H_6Cl_2N_2O$	0
3b	I	NH_2	*93*	*210-213°C*	*387.8*	$C_7H_6I_2N_2O$	0
4b	Br	NH_2	*70*	*185°C*	*293.94*	$C_7H_6Br_2N_2O$	0
1c	CH_3O	*H*	*56*	*116-119°C,*	*181*	$C_9H_{11}NO_3$	10
2c	Cl	*H*	*60*	*107-109°C*	*190*	C_7H_5ClNO	8

CONCLUSION

Among compounds which present the oxime group on the aromatic ring, those with a hydroxyl group (**1a-3a**), and those with H atom (**1c, 2c**) in 2 positions showed some modest inhibitory activity. If the substituent in 2 positions is an amino group (**1b-4b**), independently from the halosubstituent, the compound was not able to inhibit the ET_A receptors; this may suggest the importance of carbonyl

group of carboxylic and the ionization extent in the interaction with allosteric site, no advantage obtained by changing the hydroxyl group of salicylic acid to amino group.

EXPERIMENTAL SECTION

General methods: Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Infrared (IR) spectra for comparison of compounds were recorded on a Mattson 1000 FTIR spectrometer. Nuclear magnetic resonance (1HNMR) spectra were recorded on a Varian Gemini 200(200MHz) in a Ca %solution of $CDCl_3$ or DMSO-d_6 for all compounds. Peak positions are given in parts per million (ppm, δ units). The proton magnetic resonance assignments were established on the basis of the expected chemical shifts and the multiplicity of the signals. Mass spectra were recorded on a HP-5988 Spectroscopy using direct injection probe and an electron beam energy of 70eV. Reactions were routinely monitored by Thin-layer Chromatography (TLC) on 0.25mm silica gel plates (Merck 60F254)and Hydroxamic acids were visualized with $FeCl_3$ aqueous solution. Flash chromatography or Preparative Medium Pressure Liquid Chromatography (MPLC) were carried out through glass columns containing 40-63 μm silica gel (Machinery-Nagel Silica Gel 60). The MPLCs were performed using a chromatography apparatus consisting of a Buchi 681 pump, a Knauer differential refractometer detector and a Philips PM 8220 pen recorder. Solvents and reagents were obtained from commercial sources in the appropriate grade and were used without further purification unless otherwise indicated. Element Analysis was carried out by our analytical laboratory and was consistent with theoretical values to within ± 0.4%.

3, 5-Diiodosalicyladoxime, 1a; A solution of hydroxylamine hydrochloride (150.6mg, 2.168mmol) in a little amount of water (1ml) was added to 3, 5-diiodosalicylaldehyde (**1**, 500 mg, 1.337 mmol) dissolved in ethanol (19.2ml); the reaction has been kept under reflux for 3 hours and controlled by TLC. After this time, the reaction mixture was filtered, and the crude solid residue was collected. Pure **1a** has been obtained by recrystallization ($CHCl_3$). ^{1}HNMR :(DMSO), δ 7.77(d, J= 2Hz, 1H aromatic proton), 7.99(d, J= 2Hz, 1H aromatic proton), 8.31(s, CH=N), 11.15(s, OH aromatic hydroxyl group), 11, 93(s, OH oxime hydroxyl group). MS: m/z = 389(M^+, base peak), 343,244,189, 164,127, 62. (C, H, N) calculated (21.59, 1.28, 3.68), analytical (21.57, 1.27, 3.7).

3, 5-Dibromosalicyldoxime, 2a; by using the same procedure described in compound **1a,** hydroxylamine hydrochloride (150.6 mg, 2.16 mmol) in water (1.9ml), 3, 5-dibromosalicylaldehyde (**2**, 500 mg, 1.78 mmol) in ethanol (19.5 ml) , reaction mixture stirred at r.t. for one hour, recrystallization ($CHCl_3$) gave pure **2a**. ^{1}HNMR :(CD_3OD), δ, 8.21(s, CH=N), 7.63(d, J=2.4Hz, 1H aromatic), 7.46(d, J= 2.4Hz, 1H aromatic). MS, :m/z= 293(M^+),277(base peak),249,223, 196,170,143,88,62. (C, H, N), calculated (28.6, 1.7, 4.77), analytical (28.1, 1.66, 4.71).

3, 5-Dichlorosalicyldoxime, 3a; by using the same procedure described in compound **1a,** hydroxylamine hydrochloride (200.6 mg, 5.17 mmol) in water (1.9 ml) ,3, 5-dichlorosalisyldehyde (**3**, 500 mg, 3.6 mmol) in ethanol (19.2ml), gave pure **3a**.^{1}HNMR: (CD_3OD), δ, 8.24(s, CH=N), 7.371(d, J= 2.4 Hz, 1 H aromatic), 7.30(d, J= 2.4Hz, 1H aromatic).MS: m/z, 205(M^+), 187(base peak), 159, 133, 124, 97, 88, 62. (C, H, N) calculated (40.89, 2.43, 6.81), analytical (41.5, 2.5, 6.6).

2-Amino 3, 5 dimethylbenzaldehyde, 10; $LiALH_4$ (344.73mg, 9.09mmol) was added to a solution of 3, 5-dimethylanthranilic acid (**4**, 1.0 g, 6.06 mmol) in anhydrous THF (30 ml) stirred at 0°C degree under nitrogen atmosphere. The resulting mixture was stirred at room temperature one hour and then under reflux 30 min. After this time, the reaction mixture was cooled and, a mixture of THF: Water (2:1, 5ml) was added to finish the reaction, filtered, and to the filtrate MnO_2 (670.8 4mg, 18 mmol) was added; the reaction has been left under reflux for two days. The organic phase, dried and evaporated afforded the crude aldehyde which was purified by flash chromatography (Hexane/Ethyl Acetate 80:20) to give pure (**10**) .Yield= 80%, ^{1}HNMR (DMSO): δ 9.94 (s, 1H, CHO), 7.26 (s, 1H, aromatic), 7.18 (s, 1H, aromatic),

6.15 (brs, 2H, NH_2), 2.36 (s, 3H, CH_3), 2.24 (s, 3H, CH3).

2-Amino-3, 5-dimethylbenzaldoxime, 1b; A solution of hydroxylamine hydrochloride (407.21 mg, 5.86 mmol) in little amount of water (1.9 ml) was added drop-wise to a solution of 2-amino-3, 5-dimethylbenzaldehyde (**10**, 438 mg, 2.93 mmol) in ethanol (19 ml) in the presence of K_2CO_3 (809.91 mg, 5.86mmol). The reaction has been left under reflux for 4 hours, filtered, extracted with Et_2O, and the organic layers dried and evaporated afforded a solid residue which was recrystallized ($CHCl_3$) to give pure **1b**.^{1}HNMR (DMSO): δ 8.21 (s, 1H, CH=N), 6.91 (s, 1H, aromatic), 6.82 (s, 1H, aromatic), 2.22 (s, 3H, CH_3), 2.16 (s, 3H, CH_3). MS: m/z= 164(M^+, base peak), 165(M^{+1}), 147, 132, 120. (C, H, N) calculated (65.8, 7.3, 17), analytical (65.4, 7.25, 16.8).

2-Amino-3, 5-dichlorobenzaldehyde, 11; by using the same procedure described in compound **10,** $LiALH_4$ (110.44mg, 2.9mmol), 3, 5-dichloroanthranilic acid (**5**, 400mg, 1.94 mmol) in anhydrous THF (30 ml), MnO_2 (400mg, 10.82mmol), give pure (**11)**. Yield= 85%, ^{1}HNMR ($CDCl_3$): δ 9.80 (s, 1H, CHO), 7.44 (d, 1H, J=2.4 Hz aromatic), 7.41 (d, 1H, J=2.4 Hz aromatic), 6.61 (br s, 2H, NH_2).

2-Amino-3, 5-dichlorobenzaldoxime, 2b; by using same procedure described in compound **1e,** hydroxylamine hydrochloride (136.98 mg, 2 mmol) in water (1 ml), 2-amino-3,5-dichlorobenzaldehyde (**11**, 190mg, 1mmol) in ethanol (19 ml), K_2CO_3 (276 mg, 2mmol), gave pure **2b**.^{1}HNMR ($CDCl_3$): δ 8.15 (s, 1H, CH=N), 7.27 (d, 1H, J=2.4Hz, aromatic), 7.03 (d, 1H, J=2.4, aromatic), 6.01 (brs, 2H, NH_2). MS: m/z; 205(M^+), 206(M^{+1}), 204(base peak), 188, 174. (C, H, N) calculated (40.9, 2.92, 13.65), analytical (40.6, 2.89, 13.2).

2-Amino-3, 5-diiodobenzaldehyde, 12; by using the same procedure described in compound **10,** LiALH4 ((196.89mg, 5.19mmol), 3, 5-diiodoanthranilic acid (**6**, 1 g, 3.461mmol) in anhydrous THF (30 ml), MnO_2 (455mg, 12.32mmol), gave pure (**12**). Yield= 90%, ^{1}HNMR ($CDCl_3$): δ 9.67 (s, 1H, CHO), 8.10 (d, 1H, J=2Hz, aromatic), 7.98 (d, 1H, J=2Hz, aromatic), 7.14 (br s, 2H, NH_2).

2-Amino-3, 5-diiodobenzaldoxime, 3b; by using the same procedure described in compound **1e,** hydroxylamine hydrochloride (100mg , 0.7mmol) in water (1 ml) ,2-amino-3,5-diiodobenzaldehyde (**12**, 131mg, 0.352mmol) in ethanol (19 ml), K_2CO_3 (97.29mg, 0.704mmol), gave pure **3b**.^{1}HNMR (DMSO): δ 11.37 (s, 1H, OH), 8.19 (s, 1H, CH=N), 7.83 (d, 1H, J= 2 Hz, aromatic), 7.58 (d, 1H, J= 2Hz , aromatic), 6.61 (brs, 2H, NH_2).MS: m/z; 388(M^+), 389(M^{+1}), 375(base peak), 371, 375, 261, 244, 134. (C, H, N) calculated (21.6, 1.55, 7.2), analytical (21.9, 1.58, 7.2).

2-Amino-3, 5-bromobenzaldoxime, 4b; by using the same procedure described in compound **1b,** hydroxylamine hydrochloride (399mg , 5.7mmol) in (1 ml) 2-amino-3,5-dibromobenzaldehyde (**13**, 500mg, 1.79mmol) in ethanol (19 ml), K_2CO_3 (397.35mg, 2.87mmol), gave pure **4b**. ^{1}HNMR: (DMSO): δ 11.43 (s, 1H, OH), 8.27 (s, 1H, CH=NH), 7.59 (d, 1H, J=2.2Hz, aromatic), 7.49 (d, 1H, J= 2.2Hz, aromatic), 6.68 (brs, 2H, NH_2).MS: m/z = 294(M^+), 295(M^{+1}), 278, 277(base peak), 280, 215, 198. (C, H, N) calculated (28.66, 2.04, 9.55), analytical (28.4, 1.99, 9.55).

3, 5- Dimethoxybenzaldoxime: (1c): 0.660 g of NaOH and 0.714 g of hydroxylamine hydrochloride were dissolved in little amount of water (2 ml) added to 1.000 g (6.020 mmol) of 3, 5-dimethoxybenzaldahyde **14** dissolved in 10 ml of ethanol. The mixture had been left under magnetic agitation at room temperature for 18 h before it was dried under vacuum, then diluted with water and acidified to pH = 5 with HCl 1N , precipitate was filtered, collected and dried. The white solid was purified by recrystallization from EtoAc/Hexane to give pure **1c.** m.p: 116-119°C, M.W: 181. IR: 1567 cm^{-1} (C=N).^{1}H-NMR (DMSO): δ 11.23(s,1H,OH) , 8.05(s,1H,CH=N), 6.75(s,2H,Ar), 6.50(s,1H,Ar), 3.74(s,6H,2OCH_3). M.S: m/z, 181(M^+, base peak), 182(M^{+1}), 164(-OH).Elemental analysis: (C, H, N) calculated (59.66, 6.07, 7.73),analytical (59.00, 6.04, 7.70).

3, 5- Dichlorobenzaldoxime: (2c): By using the same procedure described in compound **1c,** 0.120 g (3 mmol)

of NaOH and 0.135 g of hydroxylamine hydrochloride in 0.2 ml of water , 0.200 g (1.142 mmol) of 3, 5-dichlorobenzaldahyde **15** in 6 ml of ethanol, gave pure **2c**. m.p: 107-109°C, M.W: 190.IR: 1565, 45 cm^{-1} (C=N),^{1}H-NMR (DMSO): δ 11.67(s, 1H, OH), 8.15(s, 1H, CH=N), 7.62(s, 3H, Ar).M.S: m/z, 190(M^{+}, base peak), 191(M^{+1}), 192(M^{+2}), 154(-Cl), 146. (C, H, N) calculated (44.2, 2.3, 7.4), analytical (44.5, 2.3, 7.5).

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تثبيط فعالية الاندوثلين-1 المرتبط باليود المشع على المستقبل ETA بواسطة مشتقات الالادوكزيم

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ملخـص

اندوثلين–1 (ET-1) هو عبارة عن بيبتيد يسبب تقلص الاوعية الدموية وتتم فعاليته الفيزولوجية من خلال تحفيز بروتين خاص يسمى بروتين ج الذي يرتبط بمستقبلين هما ETA و ETB و لا يغادرهما .
الاسبيرين وحامض السلسليك مركبان يثبطان عمل بيبتيد الاندوثلين (ET-1) من خلال تغير ارتباط (ET-1)بالمستقبل (ET_A) . لقد وجد أن مشتق ثنائي الهلوجين لحامض السلسليك يثبط عمل (الاندوثلين–1)خمسين مرة أكثر من الأسبرين. في هذه الدراسة تم استبدال مجموعة الكاربوكسيل بمجموعة الاوكزيم واستبدال مجموعة الهيدروكسيد بمجموعة الامين أو الهيدروجين. ثم تمت دراسة فعالية هذه المركبات الجديدة من خلال تثبيط فعالية ارتباط الاندوتلين-1 المرتبط باليود المشع (^{125}I – ET-1) بالمستقبل (ET_A). أظهرت الدراسة أنها أقل فعالية من مشتقات حامض السلسليك في تثبيط عمل ببتيد الاندوثلين-1 (ET-1) كما ورد في الأدبيات.

الكلمـات الدالـة: الدوستريك، ET_A، الدوكسيم.

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