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Synthesis and investigation of antihypertensive activity using anaesthetized-normotensive nonhuman primates of some 2-aryl-4-(substituted) pyrimido [*1,2-a*] benzimidazoles

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ABSTRACT

*In this work, we studied the in vivo pharmacological effects of the new derivatives related to fused [6-5-6] system, pyrimido-benzimidazole and compared with those of tolazoline. In anaesthetized normotensive dogs, both **6d** and tolazoline (10^{-3} mmole/kg, iv) caused a pronounced fall in mean blood pressure as hypotensive agents. Upon investigating the antihypertensive activity of **6d**, it markedly inhibited the hypertensive effect of noradrenaline. The required new pyrimido [l,2-a] benzimidazole derivatives were synthesized from precursors **1-3**. Compounds **1-3** intern obtained from condensing 2-aminobenzimidazole with beta-diketones which were then condensed with the appropriate aldehyde **4a-f** to produce three series: **5a-f, 6a-f and 7a-f**. The products are obtained in moderate yields and are in trans [E]-configuration. The structure of the synthesized derivatives has been characterized by elemental microanalysis (CHN), IR and NMR Spectroscopy, as well as physicochemical properties. The geometry of the alkene resulted from condensation was verified by IR. In conclusion: Pyrimido-benzimidazole scaffold is a good target for further search for antihypertensive agents.*

Keywords: Hypotensive activity, Anaesthetized-normotensive dogs, Pyrimido-benzimidazoles, Regioselectivity, E-Configuration.

INTRODUCTION

The antihypertensive activity of guanidine congeners; a structural feature recognized and optimized for their neuronal receptor binding in several drugs. The binding was generally enhanced by the hydrophobic ring attachments [1]. For revealing such activity, it was irrelevant to devote this to the synthesis of pyrimido [*1,2-a*] benzimidazole ring system which was recognized in literature since 1937 [2]. Several biological activities were reported such as antimicrobial [3- 5], antiviral [6], antiulcer [7], analgesic and antinflammatory [8], central nervous system active agents [9,10], antioxidants [11, 12] as well as antihypertensive [13] activities. Reports also were about pyrimido-benzimidazoles as potential antineoplastic agents [14-17]. In this report we describe the synthesis and assessement of the antihypertensive and hypotensive effects of a series of 4-substituted-ethenyl-2-aryl-pyrimido [1,2-a] benzimidazoles

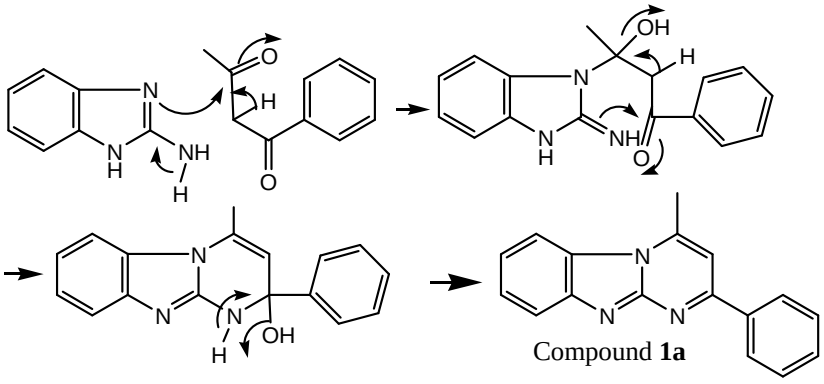
As a starting point for the synthesis, these derivatives were designed for pringing about the following structural modifications: a)-increasing the general lipophilic character of the parent molecule by insersion of a substituted ethenyl (vinylene) function at the expense of the 4-methyl group. b)-Augmenting the structural requirements found in tolazoline. c)-Ensuring possible electronic interactions by different substituents in the 2-aryl group. d)-Exploring the effect of isosteric replacement of the phenyl of 4-(un)substitutedphenyl-ethenyl group with furyl and thienyl ring structures on the hypotensive activity, if any.

RESULTS AND DISCUSSION

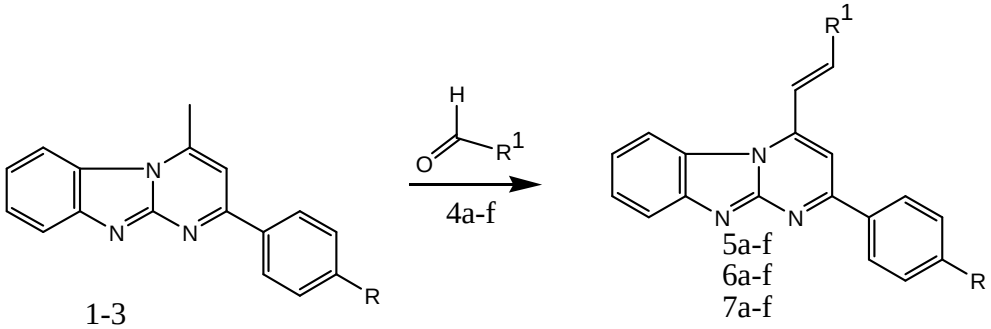
Chemistry:

The substituted benzoylacetones required for this research were prepared under the conditions of Claisen reaction [18-21]. Equimolar amounts of 2-aminobenzimidazole and the appropriate benzoylacetone derivatives are heated at 150-170 ^{0}C for 5 hr. [13]. The use of unsymmetrical beta-dicarbonyl compounds in such annelation reactions can, in theory, lead to two possible products, though in practice one isomer is often formed largely in preference to the other [2b]. In this context, thermal fusion of 2-aminobenzimidazole with benzoyl-acetone offered regioselectively 2-aryl-4-methyl-pyrimidobenzimidazole [13] (**Scheme 1**). Some 2-aryl-4-methyl-pyrimido-benzimidazoles were synthesized and claimed with appreciable hypotensive activity. SAR study denoted effective operation of both σ-electronic parameter and π-solubility parameters for this study [13].

Scheme 1: Synthetic pathway for compound 1a



Scheme 2: Synthetic pathway for compounds 5a-f, 6a-f & 7a-f



5a-f R = H, a (R^1= C_6H_5-); b (R^1= p-Cl-C_6H_4-); c (R^1= p-CH_3O-C_6H_4-);
d (R^1= p-O_2N-C_6H_4-); e (R^1= 2-furyl); f (R^1= 2-thienyl)

6a-f R = Cl, a (R^1= C_6H_5-); b (R^1= p-Cl-C_6H_4-); c (R^1= p-CH_3O-C_6H_4-);
d (R^1= p-O_2N-C_6H_4-); e (R^1= 2-furyl); f (R^1= 2-thienyl)

7a-f R =CH_3, a (R^1= C_6H_5-); b (R^1= p-Cl-C_6H_4-); c (R^1= p-CH_3O-C_6H_4-);
d (R^1= p-O_2N-C_6H_4-); e (R^1= 2-furyl); f (R^1= 2-thienyl)

Synthesis of the target compounds was achieved by condensing 4-methyl-2-(p-substituted phenyl) pyrimido-benzimidazole with various (un)substituted-benzene, furan and thiophene carbaldehyde derivatives under proper catalysis (**Scheme 2**). The rate limiting step in such a reaction is associated with generation of a carbanion from the 4-methyl group on account of its vicinity (α) to the electron-deficient bridgehead nitrogen atom. The second fast step involves attack of the carbonyl-carbon of the aldehyde component to give ultimately the ethylenic derivative. Comparative study with the spectra of the precursor compounds easily revealed the disappearance of the methyl group at C-4.

For most of the synthesized ethenyl derivatives, compounds (**5a-f, 6a-f, 7a-f, Tables 1-3**) the assignment of the configuration of the olefinic linkage could not be established by NMR because of location of its signals in the region of absorption of the aromatic protons. However, trans configuration of these compounds can be suggested on the basis of their IR spectra on account of the presence of strong absorption at 850-980 cm^{-1} [22]

Pharmacology:
All of the synthesized derivatives were tested for their hypotensive effect in anaesthetized normotensive dogs [13, 24, 25]. The calculated weight of each compound was dissolved in 3 mL of a solution [composed of (v/v) ethanol and dimethylformamide in (3:1) ratio], then completed to 20 mL with 50% aqueous ethanol so as to afford 10^{-3} molar test solutions. Adult healthy male dogs (8-10 kg) were used as provided by the department of pharmacology. Urethane (ethyl carbamate, Aldrich Chemical Company, USA) was used as 25% solution in water. Pure reference standard clonidine hydrochloride (Sigma Chemical Company, USA) was used as a reference antihypertensive agent in the same molar concentration as used for the tested compounds (10^{-3} mmole/kg). The animals were injected i.v. with a dose of 1 mL of the prepared solution per kg. animal body weight (10^{-3} mmole/kg). Results are recorded directly (D) and every few minutes for a period of 1 hour (**Tables 4-8**). The hypotensive activity of the individual derivatives increases when passing from series **5a-f** through series **7a-f**. This finding agree with the same order recorded for the hypotensive activity of the parent **1-3** derivatives [14]. It should be also noted that the most active derivative (**6d**); 2-p-chlorophenyl-4-(2'-(m-nitrophenyl) ethenyl-pyrimido[1,2-a] benzimidazole. It was of comparable activity with that o tolazoline in the same molar ratio. Another observation should be noted is that the p-methoxyphenyl analogs (**5c, 6c and 7c**) are generally characterized by lower activity, while the p-chloro and m-nitro-phenyl derivatives are of higher activity. Specific significance of the role of the m-nitro group for better fitting with relevant receptor sites was reported for clonidine nitro analogs with much emphasis on the respective influence on the receptor site in contrast to the o- or p-substituent [23].

MATERIALS AND METHODS

2-Aminobenzimidazole and p-(un)substituted benzoylacetone derivative that required as starting materials in this paper are prepared according to reported procedures [18-21]. Melting points were determined on an electro-thermal melting point apparatus [Stuart Scientific, UK], and are uncorrected. Precoated silica gel plates (kiesel gel 0.25 mm, 60G F254, Merk) were used for thin layer chromatography. Developing solvent system of chloroform/methanol (10:3) was used and the spots were detected by ultraviolet light; Spectroline ENF-240C/F (model CM-10) at short wavelength (λ = 254 nm) and/or iodine stain. All chemical yields are unoptimized and generally represent a single experiment. IR spectra (KBr disc) were recorded on IR-470 Shimadzu spectrometer, Japan. ^{1}H-NMR Spectra were scanned on a Varian EM-360 L NMR spectrometer (60 MHz) USA at Faculty of Pharmacy Assiut University. Chemical shifts are expressed in δ-values (ppm) relative to TMS as an internal standard, using $CDCl_3$ as a solvent. The microanalysis for C, H and N were performed on a Perkin Elmer 240 elemental analyzer, and were performed at the Department of Chemistry, Faculty of Science. Pharmacological screening was carried out at the department of pharmacology, faculty of medicine, El-Minia University, Al-Minia, Egypt.

1. Synthesis of the beta-diketone intermediates [18-21]
Beta-diketones required for the synthesis of precursors (**1-3)** were synthesized according to the synthetic pathway depicted in **Scheme 1** and in accordance to the previously reported procedure in reference [18-21]. The diketones were prepared using the Claisen condensation procedure by condensing acetophenone, p-methyl or p-chloro-analogue [10 mmol] and ethylacetate [10 mmol] in the presence of sodium ethoxide in dry ethanol.

2. Synthesis of precursors (1-3) [13].
When R=CH_3 (for precursor **3**) the condensation was carried out by fusion of 2-aminobenzimidazole (0.01 mole) with benzoylacetone derivatives (0.01 mole) at 150-170 ^{0}C for 5 hr. The reaction mixture was cooled, and the HCl

(25 mL, 10%). The resulting solution was carefully washed with ether (2x10 mL), and rendered alkaline by ammonia. Product is purified by chromatography. Yield 33-50% [13]

3. Synthesis of 5a-f, 6a-f, 7a-f:

A mixture of the appropriate 4-methyl-2-(p-(un)substitutedphenyl) pyrimido[1,2-a] benzimidzole (**1-3**) (5 mmole) and the appropriate carbaldehyde (5 mmole) was refluxed in glacial acetic acid (20 mL) for 24 h. The reaction mixture was cooled and poured into ice-cooled water. The formed precipitate was filtered off and crystallized from the proper solvent (**Tables 1-3**).

Table 1: The physicochemical data of 5a-f

Compd No.	R	R^1	Yield %	m.p.°C	Formula (M. Wt)	Microanalysis		
							Calcd	Found
5a	H	C_6H_5-	43	187-90 acetic acid	$C_{24}H_{17}N_3$ (347.42)	C H N	82.97 4.93 12.09	82.30 4.98 12.41
5b	H	*p*-Cl-C_6H_4-	78	198-200 Acetic acid	$C_{24}H_{16}ClN_3$ (381.86)	C H N	75.49 4.22 11.00	75.25 4.22 10.80
5c	H	*p*-CH_3O-C_6H_4-	56	220-2 ethanol	$C_{25}H_{19}N_3O$ (377.45)	C H N	79.55 5.07 11.13	79.58 5.67 10.68
5d	H	*m*-O_2N-C_6H_4-	76	275-8 Aq. ethanol	$C_{19}H_{14}N_4O_2$ (392.42)	C H N	73.46 4.11 14.28	72.77 4.65 14.02
5e	H	2-furyl	37	230-2 Aq. ethanol	$C_{22}H_{15}N_3O$ (337.38)	C H N	78.76 4.28 12.45	74.90 4.21 11.87
5f	H	2-thienyl	29	240-2 Aq. ethanol	$C_{22}H_{15}N_3S$ (353.44)	C H N	74.76 4.28 11.89	74.73 3.97 11.65

2-Phenyl-4-(2-phenylethenyl)pyrimido[1,2-a]benzimidazole; 5a: IR, cm^{-1}: 3045 (Aromatic C-H stretch), 1626, 1588, 1528 (C=C, C=N stretch), 958 (-CH=CH- trans bend), 775, 727, 691 (out-of-plane aromatic C-H bend of monosubstituted benzene ring).

2-Phenyl-4-(2-(*p*-chlorophenyl)ethenyl)pyrimido[1,2-a]benzimidazole; 5b: ^{1}H-NMR ($CDCl_3$): σ-value, ppm.: 7.30-7.70 (m, 12H, C3-H, 2H of -CH=CH-, 5H of C_6H_5, 2H of C2-H, C6-H of C_6H_4-p-Cl, 2H of C-7H, C-8-H) and 7.50-7.80 (m, 4H, C6-H, C9-H and C3-H, C5-H of C_6H_4-p-Cl), IR, cm^{-1}: 3090 (Aromatic C-H stretch), 1629, 1590, 1532 (C=C, C=N stretch), 980 (-CH=CH- trans bend).

2-Phenyl-4-(2-(*p*-methoxyphenyl)ethenyl)pyrimido[1,2-a]benzimidazole; 5c: IR, cm^{-1}: 3084 (Aromatic C-H stretch), 2985 (aliphatic C-H stretch), 1632, 1582, 1551 (C=C, C=N stretch), 975 (-CH=CH- trans bend).

2-Phenyl-4-(2-(*m*-nitrophenyl)ethenyl)pyrimido[1,2-a]benzimidazole; 5d: IR, cm^{-1}: 3115 (Aromatic C-H stretch), 1619, 1594 (C=C, C=N stretch), 1527, 1352 (NO_2), 985 (-CH=CH- trans bend).

2-Phenyl-4-(2-(2-furyl)ethenyl)pyrimido[1,2-a]benzimidazole; 5e: IR, cm^{-1}: 3090 (Aromatic C-H stretch), 1629, 1590, 1532 (C=C, C=N stretch), 980 (-CH=CH- trans bend).

2-Phenyl-4-(2-(2-thienyl)ethenyl)pyrimido[1,2-a]benzimidazole; 5f: ^{1}H-NMR ($CDCl_3$): σ-value, ppm.: 7.00-7.70 (m, 11H, C3-H, 2H of -CH=CH-, 5H of C_6H_5, 2H of C-7H, C-8-H, 1H of C5-H of thienyl) and 7.80-8.30 (m, 4H, C6-H, C9-H and C3-H, C4-H of thienyl).

Table 2: The physicochemical data of 6a-f

Compd No.	R	R^1	Yield %	m.p.°C	Formula (M. Wt)	Microanalysis		
							Calcd	Found
6a	Cl	C_6H_5-	52	242-4 DMF	$C_{24}H_{16}ClN_3$ (381.86)	C H N	75.49 4.22 11.00	75.80 4.34 10.70
6b	Cl	*p*-Cl-C_6H_4-	72	288-90 DMF	$C_{24}H_{15}Cl_2N_3$ (416.31)	C H N	69.24 3.63 10.09	69.21 4.00 10.00
6c	Cl	*p*-CH_3O-C_6H_4-	55	218-20 DMF	$C_{25}H_{18}ClN_3O$ (411.89)	C H N	72.90 4.40 10.20	72.90 4.44 10.34
6d	Cl	*m*-O_2N-C_6H_4-	73	290-2 DMF	$C_{24}H_{15}ClN_4O_2$ (426.86)	C H N	67.52 3.54 13.13	68.08 3.62 12.67
6e	Cl	2-furyl	35	237-9 ethanol	$C_{22}H_{14}ClN_3O$ (371.83)	C H N	71.07 3.79 11.30	71.41 4.02 11.00
6f	Cl	2-thienyl	40	226-8 ethanol	$C_{22}H_{14}ClN_3S$ (387.89)	C H N	68.12 3.64 10.83	68.26 3.46 10.80

2-(p-Chlorophenyl)-4-(2-phenylethenyl)pyrimido[1,2-a]benzimidazole; 6a: IR, cm^{-1}: 3105 (Aromatic C-H stretch), 1620, 1592, 1532 (C=C, C=N stretch), 978 (-CH=CH- trans bend), 770, 691 (out-of-plane aromatic C-H bend of monosubstituted benzene ring).

2-(p-Chlorophenyl)-4-(2-(p-chlorophenyl)ethenyl)pyrimido[1,2-a]benzimidazole; 6b: IR, cm^{-1}: 3100 (Aromatic C-H stretch), 1620, 1590, 1524 (C=C, C=N stretch), 980 (-CH=CH- trans bend), 770, 763, 688 (out-of-plane aromatic C-H bend of monosubstituted benzene ring).

2-(p-Chlorophenyl)-4-(2-(2-furyl)ethenyl)pyrimido[1,2-a]benzimidazole; 6e: ^{1}H-NMR ($CDCl_3$): σ-value, ppm.: 6.60 (m, 1H, C4-H, furyl), 6.75 (d, 1H, C2-H of ethenyl), 7.10-8.35 (m, 12H, C1-H of ethenyl, C3-H, C_6H_4-p-Cl, C3-H, C5-H of furyl, and C_6H_4 fused benzo.).

2-(p-Chlorophenyl)-4-(2-(2-thienyl)ethenyl)pyrimido[1,2-a]benzimidazole; 6f: ^{1}H-NMR ($CDCl_3$): σ-value, ppm.: 7.10-8.45 (m, 14H, all protons, difficult to be differentiated).

Table 3: The physicochemical data of 7a-f

Compd No.	R	R^1	Yield %	m.p.°C	Formula (M. Wt)	Microanalysis		
							Calcd	Found
7a	CH_3	C_6H_5-	58	255-7 Acetic acid	$C_{25}H_{19}N_3$ (361.45)	C H N	83.07 5.29 11.63	83.74 5.26 11.74
7b	CH_3	*p*-Cl-C_6H_4-	45	175-8 Acetic acid	$C_{25}H_{18}ClN_3$ (395.89)	C H N	75.85 4.58 10.61	75.99 4.76 10.15
7c	CH_3	*p*-CH_3O-C_6H_4-	42	210-12 Acetic acid	$C_{26}H_{21}N_3O$ (391.47)	C H N	79.77 5.41 10.73	79.46 5.00 10.64
7d	CH_3	*m*-O_2N-C_6H_4-	52	270-2 Acetic acid	$C_{25}H_{18}N_4O_2$ (406.44)	C H N	73.88 4.46 13.78	73.13 4.04 14.00
7e	CH_3	2-furyl	30	185-7 ethanol	$C_{23}H_{16}N_3O$ (350.39)	C H N	78.84 4.60 11.99	79.20 4.40 11.80
7f	CH_3	2-thienyl	23	215-7 ethanol	$C_{23}H_{16}N_3S$ (366.46)	C H N	75.38 4.40 11.46	75.70 4.8 11.32

2-(p-Tolyl)-4-(2-phenylethenyl)pyrimido[1,2-a]benzimidazole; 7a: ^{1}H-NMR ($CDCl_3$): σ-value, ppm.: 2.40 (s, 3H, CH_3), 7.20 (s,), 7.20-8.20 (16H, C3-H, -CH=CH-, C_6H_5, C_6H_4-p-subst., C_6H_4 of fused benzo).

2-(p-Tolyl)-4-(2-(p-chlorophenyl)ethenyl)pyrimido[1,2-a]benzimidazole; 7c: ^{1}H-NMR ($CDCl_3$): σ-value, ppm.: 2.45 (s, 3H, CH_3), 3.90 (s, 3H. OCH_3), 7.00-8.30 (15H, C3-H, -CH=CH-, 2xC_6H_4-p-subst., C_6H_4 of fused benzo). IR, cm-1: 3095 (Aromatic C-H stretch), 2980 (CH_3, C-H stretch), 1648, 1612, 1587 (C=C, C=N stretch), 980 (-CH=CH- trans bend).

2-(p-Tolyl)-4-(2-(2-furyl)ethenyl)pyrimido[1,2-a]benzimidazole; 7e: ^{1}H-NMR ($CDCl_3$): σ-value, ppm.: 2.40 (s, 3H, CH_3), 6.60 (m, 1H, C4-H-furyl), 7.05-8.40 (m, 12H, C3-H -CH=CH-, -C_6H_4-p-subst., C3-H & C5-H furyl, C_6H_4 of fused benzo.).

2-(p-Tolyl)-4-(2-(2-thienyl)ethenyl)pyrimido[1,2-a]benzimidazole; 7f: ^{1}H-NMR ($CDCl_3$): σ-value, ppm.: 2.45 (s, 3H, CH_3), 7.10 (s, 1H, C3-H), 7.10-8.45 (m, 12H, CH=CH-, C3-H, C4-H, C5-H of thienyl, and 4H, C_6H_4-p-subst., fused benzo C_6H_4). IR, cm^{-1}: 3070 (Aromatic C-H stretch), 2965 (CH3, C-H stretch), 1628, 1585, 1528 (C=C, C=N stretch), 960 (-CH=CH- trans bend), 730 (-C-S- bond).

Pharmacology

Procedure [13, 24, 25]:

1-Groups of dogs (each of 3 animals) were anaesthetized with an i.p. injection of urethane solutions. The right common carotid artery as well as the left jugular vein was used for intravenous injection of the drugs under investigation, while the arterial catheter was connected to a PT 400 blood pressure transducer and a CD10 amplifier of a two channel oscillograph (MD2 Bioscience, Palmer-George, Washington, USA). The transducer was first calibrated, the animal left for a thirty minute-period for stabilization and pretreatment (basal) mean blood pressure was then measured. The calculated doses of the tested compounds were injected intravenously through the jugular vein and the blood pressure was recorded directly (D) and through 1, 3, 10, 15, 30, 45 and 60 minutes following the injection of each drug (Tables **4-6**). The same procedure was performed for evaluation of the effect of the reference antihypertensive agent (**Table 7**).

2- In an another group of dogs, the solvent system was injected intravenously in the same volume used as for the tested compounds and the change in blood pressure was recorded which proved of non-significant, if ever.

3- In the third group of dogs (3 animals), norepinephrine (NE) solution (4 mg of NE bitartrate in 250 mL distilled water) was used to induce hypertension in the rate of 1.0-1.1 mL/minute. After initial elevation of blood pressure (about 1 min, considered zero time, intake of NE solution was interrupted and instead, a dose of compound (**6d**), 10^{-3} mmole/kg) was injected through the same route. Re-intake of NE solution was then continued with recording the mean blood pressure through 1, 3, 10, 15, 30, 45, and 60 minutes (**Table 8**).

Table 4: Hypotensive activity of compounds (5a-f)

C. No.	T. (min)	Dog 1			Dog 1			Dog 1			Mean %Dec.	±S.E.
		Mean B.P.	Dec.	%Dec.	Mean B.P.	Dec.	%Dec.	Mean B.P.	Dec.	%Dec.		
5a	**0**	**104**	-	-	**100**	-	-	**102**	-	-	-	
	D		52	50		44	44		55	53.9	49.3	
	3		-8	-7.7		-11	-11		-6	-6.9	-8.5	
	10		-4	-3.8		-11	-11		-4	-3.9	-6.2	
	15		-2	-1.9		-4	-4		-1	-0.9	-2.3	
	30		2	1.9		-2	-2		0	0	-1.3	
	45		2	1.9		-2	-2		1	0.9	0.3	
	60		6	5.8		-2	-2		1	0.9	1.6	2.3
5b	**0**	**102**	-	-	**98**	-	-	**100**	-	-	-	
	D		48	47.1		38	38.8		44	44	43.3	
	3		-3	-2.9		-9	-9.2		-5	-5	-5.7	
	10		0	0		-3	-2.04		-2	-2	-1.3	
	15		2	1.9		-2	-2.04		2	2	0.6	
	30		2	1.9		-2	-2.04		2	2	0.6	
	45		3	2.9		-2	-2.04		2	2	0.9	
	60		2	1.9		-2	-2.04		2	2	0.6	1.33
5c	**0**	**98**	-	-	**98**	-	-	**98**	-	-	-	
	D		42	42.9		28	28.6		42	42.9	38.1	
	3		3	3.1		-3	-3.1		1	1.2	0.3	
	10		3	3.1		2	2.04		0	0	1.7	
	15		7	7.1		2	2.04		0	0	3	
	30		2	2.04		0	0		0	0	0.7	
	45		3	3.1		0	0		0	0	1	
	60		2	2.04		0	0		0	0	0.7	0.68
5d	**0**	**98**	-	-	**98**	-	-	**101**	-	-	-	
	D		30	30.6		56	57.1		47	46.5	44.7	
	3		-4	-4.1		12	12.2		9	8.9	5.7	
	10		2	2.04		13	13.3		1	0.98	5.4	
	15		2	2.04		14	14.3		0	0	5.4	
	30		3	3.1		14	14.3		1	0.98	6.1	
	45		4	4.1		14	14.3		4	3.9	7.4	
	60		4	4.1		14	14.3		5	4.9	7.8	3.3
5e	**0**	**114**	-	-	**108**	-	-	**112**	-	-	-	
	D		56	49.1		48	44.4		54	48.2	47.2	
	3		4	3.5		5	4.6		8	7.1	5.1	
	10		2	1.8		3	2.8		6	5.4	3.3	
	15		2	1.8		9	8.3		6	5.4	5.2	
	30		10	8.8		10	9.3		7	6.3	8.1	
	45		11	9.7		9	8.3		7	6.3	8.1	
	60		12	10.5		9	8.3		7	6.3	8.4	1.2
5f	**0**	**108**	-	-	**98**	-	-	**100**	-	-	-	
	D		52	48.1		43	43.9		41	41	44.3	
	3		5	6.6		2	2		7	7	4.5	
	10		6	5.6		4	4.1		7	5	4.9	
	15		8	7.4		4	4.1		6	6	5.8	
	30		12	11.1		3	3.1		3	3	5.7	
	45		12	11.1		2	2.04		3	3	5.4	
	60		12	11.1		2	2.04		2	2	5	3.03

(C.No. = compound No., T. = Time, S.E. = standard error, Dec. = decrease), Negative values signify an increase in blood pressure

Table 5: Hypotensive activity of compounds (6a-f)

C. No.	T. (min)	Dog 1			Dog 1			Dog 1			Mean %Dec.	±S.E.
		Mean B.P.	Dec.	%Dec.	Mean B.P.	Dec.	%Dec.	Mean B.P.	Dec.	%Dec.		
6a	**0**	95	-	-	102	-	-	103	-	-	-	
	D		16	16.8		90	88.2		78	75.7	60.2	
	3		7	7.4		11	10.8		5	4.9	7.7	
	10		8	8.4		10	9.8		7	6.8	8.3	
	15		8	8.4		12	11.8		6	5.8	8.7	
	30		4	4.2		5	4.9		4	3.9	4.5	
	45		2	2.1		5	4.9		3	2.9	3.3	
	60		3	3.2		2	1.9		1	0.97	2	0.65
6b	**0**	104	-	-	101	-	-	94	-	-	-	
	D		56	53.8		61	60.4		74	78.7	64.4	
	3		1	0.9		3	2.9		6	6.4	3.4	
	10		7	6.7		7	6.9		12	12.8	8.8	
	15		7	6.7		7	6.9		12	12.8	8.8	
	30		8	7.7		5	4.9		13	13.8	8.8	
	45		9	8.7		6	5.9		14	14.9	9.8	
	60		6	5.8		7	6.9		10	10.5	7.7	1.4
6c	**0**	97	-	-	101	-	-	100	-	-	-	
	D		32	32.9		69	68.3		72	72	58.1	
	3		5	5.2		4	3.9		9	9	6	
	10		3	3.1		4	3.9		5	5	4	
	15		4	4.1		2	1.9		0	0	2	
	30		3	3.1		1	0.9		5	5	3	
	45		6	6.2		3	2.9		7	7	5.4	
	60		3	3.1		0	0		6	6	3	1.7
6d	**0**	98	-	-	98	-	-	99	-	-	-	
	D		58	59.2		56	57.1		58	58.6	58.3	
	3		9	9.2		10	10.2		9	9.1	9.5	
	10		11	11.2		15	15.3		11	11.1	12.5	
	15		8	8.2		10	10.2		16	16.2	11.5	
	30		19	19.4		21	21.4		20	20.2	20.3	
	45		22	22.5		24	24.5		23	23.2	23.4	
	60		22	22.5		24	24.5		23	23.2	23.4	0.59
6e	**0**	98	-	-	97	-	-	97	-	-	-	
	D		46	43.4		63	61.8		12	12	39.1	
	3		4	4.1		7	7.2		19	19.6	10.3	
	10		8	8.2		4	4.1		7.2	6.5	6.5	
	15		12	12.2		5	5.2		6.2	7.9	7.9	
	30		11	11.2		3	3.1		0	0	4.8	
	45		6	6.1		5	5.2		0	0	3.8	
	60		6	6.1		6	6.2		-1	-1.03	3.8	2.4
6f	**0**	106	-	-	102	-	-	100	-	-	-	
	D		46	43.4		63	61.8		12	12	39.1	
	3		20	18.9		13	12.8		2	2	11.2	
	10		6	5.6		3	2.9		4	4	4.2	
	15		8	7.5		1	0.98		5	5	7.2	
	30		6	5.6		2	1.9		2	2	3.2	
	45		6	5.6		1	0.98		2	2	2.9	
	60		6	5.6		1	0.98		2	2	2.9	1.4

(C.No. = compound No., T. = Time, S.E. = standard error, Dec. = decrease), Negative values signify an increase in blood pressure

Table 6: Hypotensive activity of compounds (7a-f)

C. No.	T. (min)	Dog 1			Dog 1			Dog 1			Mean %Dec.	±S.E.
		Mean B.P.	Dec.	%Dec.	Mean B.P.	Dec.	%Dec.	Mean B.P.	Dec.	%Dec.		
7a	**0**	**96**	-	-	**96**	-	-	**98**	-	-	-	
	D		20	20.8		34	35.7		40	40.8	32.3	
	3		-6	-6.3		-6	-6.3		4	4.1	-2.8	
	10		7	7.3		2	2.1		6	6.1	5.2	
	15		7	7.3		5	5.2		6	6.1	6.2	
	30		4	4.2		12	12.5		14	14.3	10.3	
	45		4	4.2		12	12.5		13	13.3	10	
	60		4	4.2		14	14.6		14	14.3	11	3.4
7b	**0**	**96**	-	-	**96**	-	-	**96**	-	-	-	
	D		40	41.7		58	60.4		79	82.3	61.5	
	3		6	6.3		6	6.3		2	2.1	4.6	
	10		-2	-2.1		-2	-2.1		-2	-2.1	-2.1	
	15		0	0		2	2.1		0	0	0.7	
	30		14	14.6		10	10.4		8	8.3	11.1	
	45		16	16.7		6	6.3		8	8.3	10.4	
	60		14	14.6		6	6.3		15	15.6	12.2	2.9
7c	**0**	**110**	-	-	**107**	-	-	**106**	-	-	-	
	D		11	10		17	15.9		31	29.2	24.3	
	3		3	2.7		2	1.9		11	10.4	5	
	10		12	10.9		9	8.4		8	7.6	8.9	
	15		12	10.9		9	8.4		8	7.6	8.9	
	30		5	4.5		5	4.7		6	5.7	4.9	
	45		1	0.9		6	5.6		8	7.6	4.9	
	60		0	0		7	6.5		6	5.7	4	2.0
7d	**0**	**98**	-	-	**106**	-	-	**104**	-	-	-	
	D		17	17.5		26	24.5		32	30.8	24.3	
	3		3	3.6		4	3.8		5	4.8	3.9	
	10		5	5.1		4	3.8		3	2.9	4.9	
	15		4	4.1		5	4.7		3	2.9	3.9	
	30		4	4.1		3	2.8		0	0	2.3	
	45		4	4.1		3	2.8		1	0.96	2.9	
	60		4	4.1		2	1.9		2	1.92	2.6	0.73
7e	**0**	**102**	-	-	**102**	-	-	**101**	-	-	-	
	D		34	33.3		38	37.3		35	34.7	35.1	
	3		11	10.8		23	22.6		15	14.8	12.1	
	10		12	11.8		14	13.7		-3	-2.9	7.5	
	15		18	17.6		24	23.5		5	4.9	15.2	
	30		17	16.7		25	24.5		16	15.5	19	
	45		22	21.6		22	21.6		18	17.8	20.3	
	60		12	11.8		7	6.9		10	9.9	9.5	1.4
7f	**0**	**103**	-	-	**108**	-	-	**114**	-	-	-	
	D		57	55.3		27	25		23	20.2	33.5	
	3		9	8.7		4	3.7		-3	-2.6	3.3	
	10		-8	-7.8		9	8.3		6	5.3	3	
	15		-11	-10.7		17	15.7		6	5.3	1.9	
	30		-2	-1.9		4	3.7		7	6.1	2.6	
	45		0	0		4	3.7		6	5.3	3	
	60		2	1.9		4	3.7		6	5.3	3.6	0.98

(C.No. = compound No., T. = Time, S.E. = standard error, Dec. = decrease), Negative values signify an increase in blood pressure

Table 7: The hypotensive activity of the reference clonidine hydrochloride under the same conditions

C. No.	T. (min)	Dog 1			Dog 1			Dog 1			Mean %Dec.	±S.E.
		Mean B.P.	Dec.	%Dec.	Mean B.P.	Dec.	%Dec.	Mean B.P.	Dec.	%Dec.		
	0		-	-		-	-		-	-	-	
	D		-1	-1.03		0	0		0	0	-0.3	
	3		-13	-13.4		-15	-15		-26	-26	-17.8	
	10	**97**	12	12.4	**100**	4	4	**104**	3	2.9	6.4	
	15		17	17.5		34	24		14	14.5	18.7	
	30		17	17.5		14	14		22	21.2	17.6	
	45		23	23.7		20	20		26	25	22.9	
	60		26	26.8		19	19		26	25	23.6	2.4

(C.No. = compound No., T. = Time, S.E. = standard error, Dec. = decrease), Negative values signify an increase in blood pressure

Table 8: The antihypertensive activity of compound 6d

C. No.	T. (min)	Dog 1			Dog 1			Dog 1			Mean %Dec.	±S.E.
		Mean B.P.	Dec.	%Dec.	Mean B.P.	Dec.	%Dec.	Mean B.P.	Dec.	%Dec.		
	0		-	-		-	-		-	-	-	
	D		28	18.7		22	14.7		40	26.7	20	
	3		32	21.3		30	20		40	26.7	22.7	
6d	10	**151**	32	21.3	**150**	33	22	**148**	32	21.3	21.5	
	15		32	21.3		30	20		32	21.3	20.9	
	30		42	28		26	17.3		32	21.3	22.2	
	45		42	28		26	17.3		32	21.3	22.2	
	60		42	28		27	18		32	21.3	22.4	2.9

(C.No. = compound No., T. = Time, S.E. = standard error, Dec. = decrease), Results of epinephrine-hypertensive animals.

CONCLUSION

In conclusion, the results obtained in this study strongly suggest that pyrimido-benzimidazole target can be considered as a good scaffold for antihypertensive activity, without the initial elevation in BP whin the 5 minutes following the iv injection of tolazoline. This finding can allow for further investigations and modifications.

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