ELSEVIER

Il Farmaco 57 (2002) 543–548

IL FARMACO

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Synthesis and effects on intracellular calcium of some 1,3-bis-(heteroaryl substituted)benzene derivatives

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Received 3 December 2001; accepted 4 December 2001

Abstract

A series of 1,3-bis-(heteroaryl substituted)benzene derivatives was designed as promising molecules which might increase intracellular Ca^{2+} level in F2408 fibroblast-like cells by affecting the Ca^{2+} channels on plasma membrane. Mentioned compounds were obtained by the treatment of isophtalaldehyde with benzil or 1,2-phenylenediamine derivatives. In this way, 13 compounds were synthesised and their structure elucidations were performed by IR, ^{1}H NMR and mass spectroscopic data and elemental analysis results. Some of the compounds showed Ca^{2+} being released from the intact cells. © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

Keywords: 1,3-Bis-(heteroaryl substituted)benzene; Intracellular calcium; Fibroblast-like cells

1. Introduction

1,2(3)(4)(5)-Bis(substituted)benzene nucleus is found in a variety of compounds which possess various pharmacological effects such as antitumour [1,2], cytotoxic [3], antiviral [4], fungicide [5,6], muscle relaxant [7], acaricide [8–10], insecticide [8,11], lipoxygenase inhibitor [12], juvenile hormone activity [9,10,13], larvacidal and mutagenic activity [14], toxicity to mosquitoes [15], insect attractiveness [16], plant growth-inhibiting [17], parasiticide [18], amebicid and trichomonacid [19] activities. Therefore, these findings prompted us to synthesise some new compounds which would be effective on intracellular calcium. In this study, some 1,3-bis(heteroaryl)benzene derivatives were synthesised and their effects on intracellular calcium were examined.

2. Chemistry

For the syntheses of 1,3-bis(heteroaryl)benzene derivatives, isophtalaldehyde and benzil or 1,2-phenylenediamine derivatives were reacted with ammonium acetate [20,21] or $NaHSO_3$ [22–24], respectively, in glacial acetic acid or alcohol (Schemes 1 and 2).

The structure elucidation of the prepared compounds was performed by IR, ^{1}H NMR and mass spectral data and elemental analyses.

3. Experimental

3.1. Chemistry

Melting points of the compounds were determined using Stuart Scientific Smpl melting point apparatus and were reported uncorrected. Thin Layer Chromatography was performed on silica gel $60F_{254}$ precoated aluminium sheet (0.2 mm) (Merck) and spots were visualised by UV lamp. IR spectra were detected in KBr pellets using a JASCO FT-IR-430 IR spectrophotometer. The ^{1}H NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 by XL-200 Varian spectrometer (400 MHz) using tetramethylsilane as an internal standard. LC-MS (EI) spectra were recorded at Platform II apparatus. Elemental analyses were performed by Carlo Erba 1106 Analyser and results for C, H, N were within $\pm 0.4\%$ of calculated values. All the chemicals and solvents used in this study were at analytical grade (Merck, Sigma and Aldrich).

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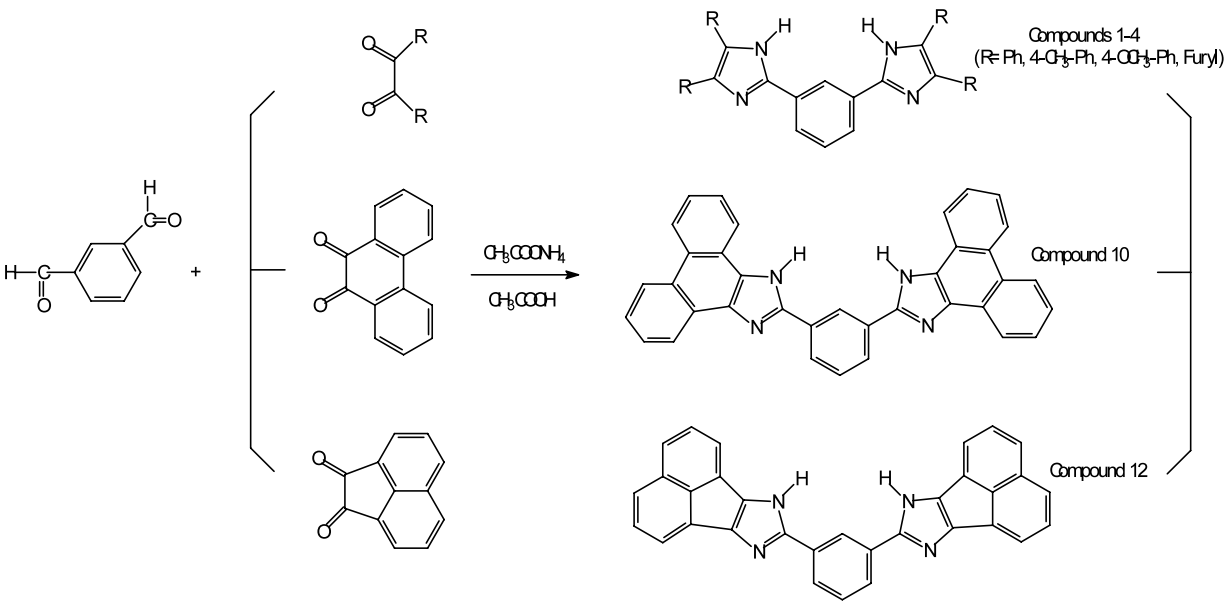
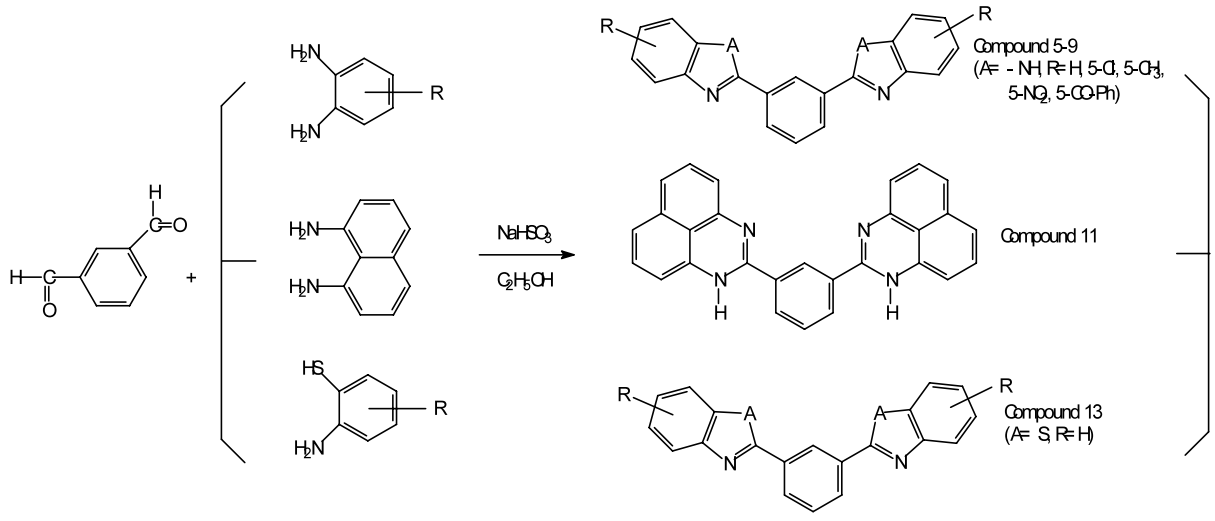
PII: S0014-827X(02)01246-6

Method A



Scheme 1.

Method B



Scheme 2.

Table 1
Structures and properties of prepared compounds

Comp. no.	Molecular formula	Synthetic method	M.W. (g/mol)	m.p. (°C)	Yield (%)	Reaction time (h)
1	$C_{36}H_{26}N_4$	A	514	188	65	3.5
2	$C_{40}H_{37}N_4$	A	570	184	62	3
3	$C_{40}H_{37}N_4O_4$	A	634	154	64	4
4	$C_{28}H_{18}N_4O_4$	A	474	240	68	3
5	$C_{20}H_{14}N_4$	B	310	148 dec.	75	3
6	$C_{20}H_{12}N_4Cl_2$	B	379	152 dec.	73	3.5
7	$C_{22}H_{18}N_4$	B	338	126 dec.	70	3
8	$C_{20}H_{12}N_6O_4$	B	400	226 dec.	69	3
9	$C_{34}H_{22}N_4O_2$	B	518	178	78	2.5
10	$C_{36}H_{22}N_4$	A	510	244 dec.	66	4
11	$C_{28}H_{18}N_4$	B	410	143 dec.	74	3
12	$C_{32}H_{18}N_4$	A	458	186 dec.	58	3.5
13	$C_{20}H_{12}N_2S_2$	B	344	180	67	3

Table 2
IR and ^{1}H NMR of the compounds

Comp.	IR (KBr, cm^{-1})	^{1}H NMR (250 MHz), (DMSO-d_6+$CDCl_3$, δ (ppm))	EI MS (m/z)
1	3624–3059 (N–H), 1603–1531 (Ar C=C and C=N), 1503–1457 (C–N), 970, 918, 898 (1,3-disubstituted), 762–694 (monosubstituted benzene) (Ar C–H).	7.10–7.12 (13H, t), 7.19 (1H, s), 7.26–7.28 (8H, d), 7.61–7.62 (2H, d), 8.43 (2H, s, –NH).	
2	3470–3023 (N–H), 1613–1519 (Ar C=C and C=N), 1494–1456 (C–N), 972, 820, 737 (1,3-disubstituted), 718 (1,4-disubstituted benzene) (Ar C–H).	2.21 (3H, s, –CH_3), 2.27 (3H, s, –CH_3), 2.41–2.42 (6H, s, –CH_3), 7.03–7.05 (4H, d), 7.15–7.17 (4H, d), 7.31–7.33 (4H, d), 7.37–7.39 (4H, d), 7.48–7.50 (2H, m), 7.95–7.97 (2H, d), 8.67 (2H, s, –NH).	570, 350 (%100), 220, 193, 178, 91, 89, 78, 77, 76, 65, 51, 38.
3	3590–3108 (N–H), 1613–1574 (Ar C=C and C=N), 1534–1462 (C–N), 1248–1174 (C–O–C), 970, 832, 802 (1,3-disubstituted), 769 (1,4-disubstituted benzene) (Ar C–H).	3.70 (12H, s, –OCH_3), 6.67–6.70 (8H, d), 7.14–7.20 (2H, m), 7.23–7.26 (8H, d), 7.65–7.67 (2H, d), 8.41 (2H, s, –NH).	
4	3618–3136 (N–H), 1608–1549 (Ar C=C and C=N), 1482–1455 (C–N), 1202–1022 (C–O–C), 885, 805, 733 (1,3-disubstituted benzene) (Ar C–H).	6.41–6.47 (1H, t), 6.77–6.79 (2H, d), 7.31–7.50 (4H, m), 7.89 (1H, s), 7.98–7.99 (4H, d), 8.00–8.01 (4H, d), 8.67–8.70 (2H, s, –NH).	
5	3486–3214 (N–H), 1623 (Ar C=C and C=N), 1490–1439 (C–N), 901, 841, 765 (1,3-disubstituted), 742 (1,2-disubstituted benzene) (C–H).	7.15–7.16 (1H, s), 7.47–7.50 (2H, d), 7.58–7.67 (5H, m), 8.18–8.19 (2H, d), 8.20 (2H, d), 8.99 (2H, s, –NH).	
6	3407 (N–H), 1623–1539 (Ar C=C and C=N), 1506–1457 (C–N), 1059 (Ar C–Cl), 927, 857, 800 (1,3-disubstituted benzene) (Ar C–H).	7.17–7.19 (2H, d), 7.53 (1H, s), 7.65–7.69 (1H, t), 8.20 (2H, d), 8.22 (2H, d), 8.60 (2H, s), 8.97 (2H, s, –NH).	
7	3374 (N–H), 1623–1558 (Ar C=C and C=N), 1539–1456 (C–N), 801, 689, 668 (1,3-disubstituted benzene) (Ar C–H).	2.36 (3H, s, –CH_3), 2.41 (3H, s, –CH_3), 6.24 (1H, s), 6.95–6.97 (2H, d), 7.93 (1H, t), 8.14–8.16 (2H, d), 8.66–8.68 (2H, d), 8.93 (2H, s, –NH)	340, 121 (%100), 104, 92, 91, 76, 65, 41.
8	3436–3347 (N–H), 1628–1596 (Ar C=C and C=N), 1518–1439 (C–N), 949, 880, 816 (1,3-disubstituted benzene) (Ar C–H).	7.30–7.33 (2H, d), 7.34 (1H, s), 7.50–7.58 (1H, t), 7.61 (2H, s), 7.98–8.01 (2H, d), 8.22–8.24 (2H, d), 8.99 (2H, s, –NH).	
9	3199 (N–H), 1644 (C=O), 1617–1574 (Ar C=C and C=N), 1534–1445 (C–N), 894, 851, 791 (1,3-disubstituted), 708, 658 (monosubstituted benzene) (C–H).	7.38–7.41 (2H, d), 7.45–7.49 (1H, t), 7.55–7.57 (2H, d), 7.64–7.65 (2H, d), 7.67–7.69 (2H, d), 7.71 (2H, d), 7.73–7.75 (2H, d), 7.81 (1H, s), 8.02 (2H, s), 8.28–8.29 (2H, d), 8.30–8.31 (2H, d), 9.10 (2H, s, –NH).	
10	3311–3071 (N–H), 1604–1591 (Ar C=C and C=N), 1460–1379 (C–N), 808, 789, 753 (1,3-disubstituted), 694 (1,2-Disubstituted benzene) (Ar C–H).	7.56–7.60 (4H, t), 7.64–7.67 (5H, t), 7.67–7.69 (2H, d), 7.74 (1H, s), 8.33 (2H, d), 8.36 (2H, d), 8.65–8.67 (2H, d), 8.68–8.70 (2H, d), 9.27 (2H, s, –NH).	
11	3347–3310 (N–H), 1600–1481 (Ar C=C and C=N), 1412–1381 (C–N), 814, 762, 668 (1,3-disubstituted benzene) (Ar C–H).	6.49–6.51 (4H, d), 6.98–7.00 (4H, d), 7.09–7.13 (4H, t), 7.36–7.40 (1H, t), 7.57–7.60 (2H, d), 7.76 (1H, s), 7.87 (2H, s, –NH).	
12	3419 (N–H), 1605–1480 (Ar C=C and C=N), 1437–1383 (C–N), 819, 771, 668 (1,3-disubstituted benzene) (Ar C–H).	7.64–7.70 (4H, t), 7.92–7.94 (2H, d), 7.95 (1H, s), 8.01–8.05 (1H, t), 8.23–8.25 (2H, d), 8.32–8.34 (2H, d), 8.49–8.51 (2H, d), 8.67–8.68 (2H, d), 8.83 (2H, s, –NH).	
13	3407 (N–H), 1590–1558 (Ar C=C and C=N), 1510–1433 (C–N), 749 (1,2-disubstituted), 959, 862, 794 (1,3-disubstituted benzene) (Ar C–H).	7.10 (1H, s), 7.23–7.27 (2H, t), 7.34–7.38 (2H, t), 7.43–7.47 (1H, t), 7.76–7.78 (2H, d), 7.96–7.98 (2H, d), 8.04–8.06 (2H, d).	

3.1.1. General procedures for the synthesis of 1,3-bis-(heteroaryl substituted)benzene derivatives

3.1.1.1. Method A. A mixture of isophtalaldehyde (0.01 mol) and benzil derivative (0.02 mol) in glacial acetic acid was reacted with ammonium acetate by the reflux for 3–6 h. At the end of the reaction, the content of the reaction vessel was poured into ice-water and neutralised with ammonia solution. The precipitate was filtered and recrystallised from ethanol. Synthesized compounds with this procedure were shown (Scheme 1).

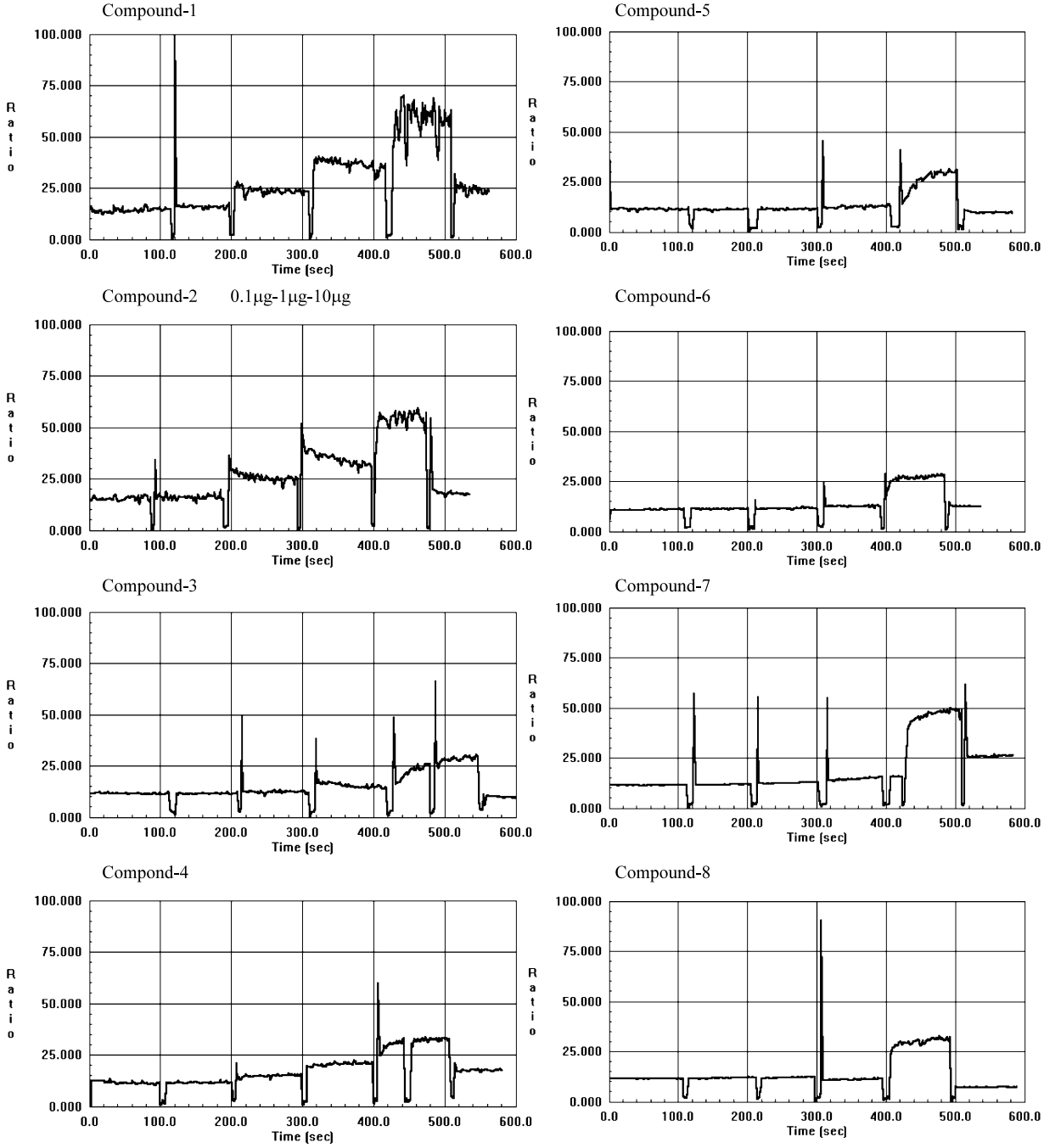
3.1.1.2. Method B. A mixture of isophtalaldehyde (0.01 mol) and 1,2-phenylenediamine derivative (0.02 mol) in alcohol was reacted with the presence of $NaHSO_3$ by the reflux for 3–4 h. At the end of the reaction, ethanol was evaporated and residue was washed with water, dried and crystallised from ethanol. Synthesized compounds with this procedure were shown (Scheme 2).

Melting points and yields are collected Table 1. Spectral data of the compounds are given in Table 2.

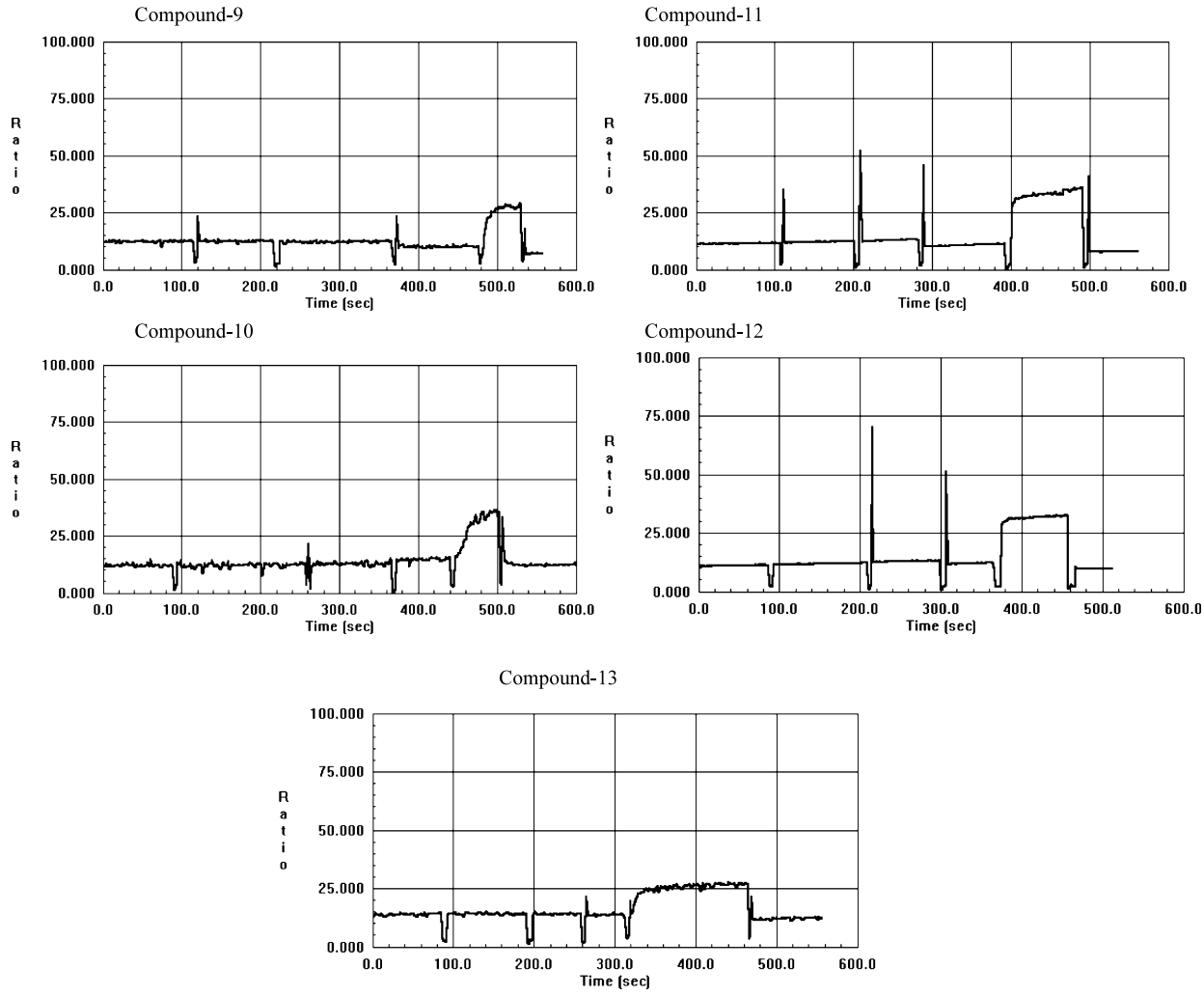
3.2. Biochemistry

3.2.1. Calcium measurement in intact cells using Fura-2

For measuring $[Ca]_i$ (intracellular calcium), cells (2×10^8) were centrifuged and resuspended in Krebs-Ringer. Cells were washed twice and resuspended at 2×10^7 cells/ml in Krebs-Ringer containing 5 μM Fura-2 AM (Sigma). Cells were loaded with Fura-2 at 37 °C for 45 min. They were washed twice by centrifugation at 1000 rpm for 5 min and resuspended in 10 ml of fresh Krebs-Ringer without Fura-2 AM and stored at 25 °C. Fura-2 fluorescence was measured using the dual excitation wavelength mode of the Shimadzu 5301-PS Spectrofluorometre.



Scheme 3.



Scheme 4.

4. Results and discussion

4.1. Chemistry

In this article, we report on the synthesis of 1,3-bis(heteroaryl)benzenes (**1**–**13**) (Schemes 1 and 2) for the first time and the results of the binding affinities for the intracellular calcium. The structures assigned to **1**–**13** were supported by IR and ^{1}H NMR analyses.

4.2. Biochemistry

The effects of 13 compounds which were selected by their structural properties were studied on intracellular calcium level. All of the compounds were examined between the concentrations of 0.1, 1 and 10 μg/ml [2,3].

Compounds **1**, **2**, **3** and **4** increased intracellular Ca^{2+} level in F2408 fibroblast-like cells by a dose-dependent (Schemes 3 and 4). These four compounds might increase the Ca^{2+} levels by affecting the Ca^{2+} channels on plasma membrane. In contrast, the other compounds have no effect on Ca^{2+} levels. No attempt has been made in this present study to describe the complex molecular mechanism through which the observed changes in the level of $[Ca]_i$ are induced. Rather these results serve as initial observations clearly showing that compounds **1**, **2**, **3** and **4** mediate a rise of intracellular Ca^{2+}. This study may be a useful basis for future investigations attempting to describe and characterise the mechanisms involved in Ca^{2+} mobilisation, specifically the nature of compound and Ca^{2+} entry pathways involved.

Acknowledgements

Part of this study was supported by the Anadolu University Research Fund (Project No. 990318).

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