Full Paper

Synthesis of Some Novel Azo Derivatives of 3,5-Dimethly-1-(2-hydroxyethyl)pyrazole as Potent Analgesic Agents

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A series of 1-(2-hydroxyethyl)-3,5-dimethylpyrazolylazo derivatives, incorporating thiosemicarbazide **2a–c**, 1,3,4-thiadiazole **3a–c**, and 1,2,4-triazole-3-thione **4a–c** were synthesized. The structure of these novel synthesized compounds **2a–c**, **3a-c**, and **4a–c** was confirmed by spectral analysis. All these compounds were screened for their analgesic activity. Hot-plate and tail-immersion tests were used for the determination of the analgesic activity. Morphine, an analgesic through both spinal and supraspinal pathways, was used as a standard test drug. All compounds were administered at a dose of 100 mg/kg *i.p*. Among the compounds, 2-(butylamino)-5-[((1-(2-hydroxyethyl)-3,5-dimethylpyrazole-4-yl)azo)phenyl]-1,3,4-thiadiazole **3a** and 4-[((1-(2-hydroxyethyl)-3,5-dimethylpyrazole-4-yl)azo)phenyl]-4-(2-phenethyl)-2,4-dihydro-*3H*-1,2,4-triazole-3-thione **4c** showed analgesic effects in both tests. Especially **4c** exerted strong analgesia starting at 30 min after injection.

Keywords: Phenylazo compounds / Pyrazoles / Thiadiazoles / Triazoles / Analgesic activity

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Introduction

Nonsteroidal anti-inflammatory and analgesic drugs are important therapeutic agents for treatment of pain and inflammation. However, prolonged use of these drugs causes gastrointestinal ulcers and, hence, there is need to develop safer analgesic and anti-inflammatory agents. Extending our interest in the search for new compounds as potent analgesic agents without side effects like ulcerogenic activity, we have synthesized a series of azo compounds carrying heterocylic rings.

Many substituted five-membered heterocycle pyrazoles, 1,2,4-triazoles and 1,3,4-thiadiazoles are known to posses a wide range of pharmacological activities, such as antibacterial [1–3], anticonvulsant [4–6], and antitubercular [7–8] activities. Moreover, the pyrazole nucleus ranks among those molecular structures related to potent analgesic activity [9–10]. In addition, thiadiazole or triazole derivatives were documented to be effective as anti-inflammatory agents [11–13]. Furthermore, although limited, there are examples in the literature on the analgesic activity of azo compounds [14–15]. Recently, a series of compounds containing pyrazole, thiadiazole, and triazole rings has been synthesized in our laboratory and the compounds have been evaluated for their encouraging antibacterial [16–19] and anticonvulsant activities [20–22]. In this series, the pyrazole moiety was connected via an azo bridge to the substituted thiadiazole or triazole moiety. The combination of different pharmacophores in one frame may lead to compounds with interesting pharmacological profiles. In view of pharmacological profiles of these three chemical moieties, as described above, we considered it interesting to further explore the biological properties of compounds **3a–c**, **4a–c** and their precursors **2a–c**. In the present work, the series of azo compounds were synthesized and tested for their analgesic activity. Two different analgesia tests were used for the determination of the analgesic activity. The hot-plate test was used for the

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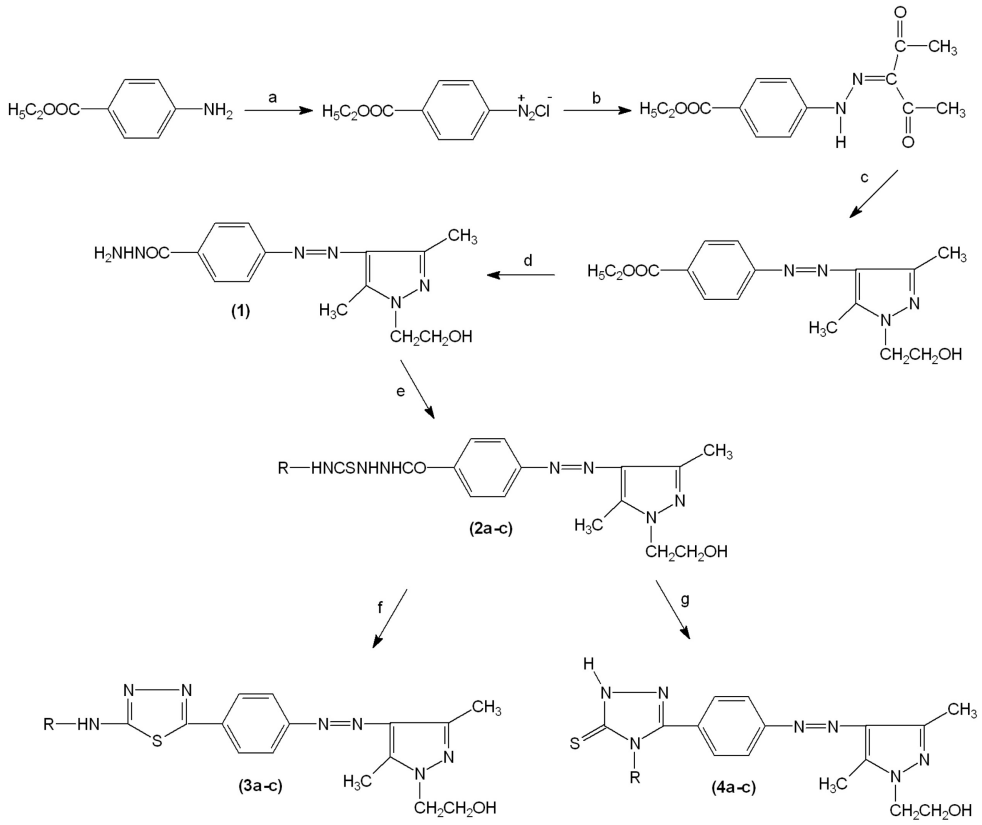

Scheme 1. Synthetic pathway for the preparation of the target molecules **1**, **2a–c**, **3a–c**, and **4a–c**. R= C_4H_9 (a), C_6H_{11} (b), $CH_2CH_2C_6H_5$ (c). Reaction conditions: (a) $NaNO_2$, HCl, 0-5 °C, (b) acetylacetone, CH_3COONa, (c) 2-hydroxyethylhydrazine, AcOH, reflux, (d) hydrazine hydrate, (e) substituted isothiocyanates, ethanol, (f) H_2SO_4 g: 2N NaOH.

supraspinal analgesia, while the tail-immersion test was used for spinal analgesia.

Results and discussion

Chemistry

The synthetic pathway followed for the preparation of the target molecules **1**, **2a-c**, **3a-c**, and **4a-c** is depicted in Scheme 1. The starting material 4-(4-ethoxycarbonylphenylazo)-1-(2-hydroxyethyl)-3,5-dimethylpyrazole was prepared according to the literature [23]. 4-(4-ethoxycarbonylphenylazo)-1-(2-hydroxyethyl)-3,5-dimethylpyrazole was reacted with hydrazine hydrate in ethanol to give the hydrazide compound **1** [23]. The hitherto unknown thiosemicarbazides **2a-c** were obtained upon the reaction of **1** with substituted isothiocyanates in ethanol. Cyclization of **2a-c** with sulfuric acid or sodium hydroxide resulted in the formation of the new thiadiazole and triazole compounds **3a–c** and **4a–c,** respectively. Physical properties and UV data of these compounds are given in Table 1.

The purity of the compounds was checked by HPLC using an diode array detector. The structures of the obtained compounds were elucidated by spectral data. The IR spectra of all compounds showed the significant bands in the expected regions. After cylication by sulfuric acid or sodium hydroxide, absence of resonances assigned to the N^1-H and N^2-H protons of the thiosemicarbazides **2a–c** provided confirmatory evidence of thiadizole **3a–c** and triazole **4a–c** formation. The other protons were observed at the expected regions. Mass spectra (MS-ES) of the compounds showed a $[M+H]^+$ peak, in agreement with their molecular formula.

Pharmacology

Two different analgesia tests were used for the determination of the analgesic activity. The hot-plate test was used for the supraspinal analgesia, while the tail-immersion test was used for spinal analgesia. Morphine, an analgesic through both spinal and supraspinal pathways, was used as a standard test drug. All compounds were administered at a dose of 100 mg/kg *i.p*. Bederson-modified neurological examination was also conducted with

Table 1. Physical properties and UV data of **1**, **2a–c**, **3a–c**, and **4a–c**.

Compound	Mp. [°C]	Yield [%]	Mol. Formula (MW)	UV, λ_{max} [nm] (log ε)
2a	190–193	65	$C_{19}H_{27}N_7O_2S$ (417)	344(4.27) 242(4.20)
2b	195–197	61	$C_{21}H_{29}N_7O_2S$ (443)	345(4.38) 243(4.31)
2c	204–206	73	$C_{23}H_{27}N_7O_2S$ (465)	368(4.43) 231(3.94)
3a	125–126	59	$C_{19}H_{25}N_7OS$ (399)	360(4.33) 233(4.01)
3b	292–293	61	$C_{21}H_{27}N_7OS$ (425)	345(4.15) 232(4.00)
3c	180–182	51	$C_{23}H_{25}N_7OS$ (447)	347(4.41) 231(4.26)
4a	193–195	93	$C_{19}H_{25}N_7OS$ (399)	345(4.42) 255(4.34) 229(4.16)[a]
4b	233–234	82	$C_{21}H_{27}N_7OS$ (425)	343(4.41) 257(4.32) 228(4.18)[a]
4c	283–284	89	$C_{23}H_{25}N_7OS$ (447)	345(4.36) 257(4.28) 228(4.11)[a]

[a] Shoulder.

the mice. No statistically significant changes in the total neurologic score were observed at these doses (Figure 1). The triazole derivative of **4c** and thiadiazole derivative of **3a** showed analgesic effects in both tests (Tables 2 and 3). Especially **4c** exerted strong analgesia starting at 30 min after injection. The analgesic effect of **4c** was close to that of morphine at 30 min. On the other hand, **3a** analgesia started at 60 min and continued to 120 min. Although **1**, **3c**, and **4a** produced an increase in hot-plate latencies, these were not statistically significant. Our results demonstrate that these compounds have significant analgesic effects in both tests indicating that these effects were mediated by both spinal and supraspinal pathways. Since our findings are preliminary results of a single dose; further studies need to be carried out to investigate the other specifications, such as dose-response and toxicological studies or side effect-activity profiles of these compounds.

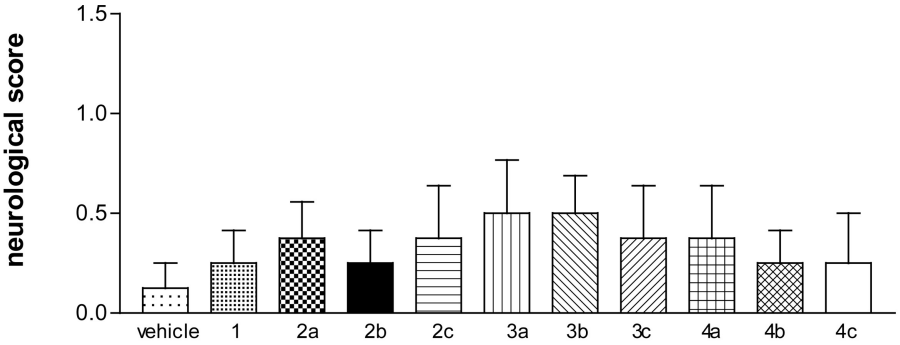

Figure 1. Neurological examination scores in Bederson's test for rats. The max for neurological examination scores in the ANOVA test.

Table 2. Hot-plate latencies of the groups.

Compound	0	30 min	60 min	90 min	120 min	150 min
1	9.51 ± 0.30	10.46 ± 1.20	10.13 ± 1.40	10.23 ± 1.80	9.79 ± 1.00	
2a	9.48 ± 0.50	9.65 ± 0.40	10.21 ± 0.90	14.40 ± 2.00	11.45 ± 1.70	
2b	9.54 ± 0.30	12.66 ± 2.30	11.54 ± 1.90	10.18 ± 1.80	9.99 ± 0.90	
2c	9.49 ± 0.50	8.99 ± 1.60	9.76 ± 0.90	9.73 ± 0.70	9.79 ± 0.50	
3a	9.50 ± 0.40	11.50 ± 0.50	12.6 ± 1.00	14.00 ± 1.00[a]	11,9 ± 0.50	10.60 ± 0.40
3b	9.35 ± 0.30	10.20 ± 0.40	9.45 ± 0.30	9.79 ± 0.40	9.33 ± 0.40	
3c	9.53 ± 0.20	11.68 ± 0.80	10.73 ± 0.60	9.76 ± 0.50	9.67 ± 0.40	
4a	9.32 ± 0.30	13.60 ± 1.80	10.70 ± 0.70	9.53 ± 0.40	9.72 ± 0.20	
4b	9.44 ± 0.40	10.90 ± 0.90	11.70 ± 1.20	9.61 ± 0.30	9.98 ± 0.40	
4c	9.55 ± 0.40	19.10 ± 2.50[a,c]	15.20 ± 1.70[a,c]	10.40 ± 1.00	10.00 ± 0.70	
Vehicle	9.49 ± 0.20	9.79 ± 0.10	9.41 ± 0.40	9.38 ± 0.40	9.94 ± 0.20	9.76 ± 0.30
Morphine	9.55 ± 0.20	19.50 ± 0.70[a,d]	24.70 ± 1.70[a,d]	18.20 ± 0.70[b]	14.1 ± 0.90	11.90 ± 0.50

The results were expressed as mean ± SEM. Repeated measures of ANOVA were performed for statistical analysis (n = 8 for each group).

[a] $p < 0.05$, [b] $p < 0.001$, when compared with its own baseline value or control group ([c]) $p < 0.05$, [d] $p < 0.01$).

Table 3. Tail-flick latencies of the groups.

Compound	0	30 min	60 min	90 min	120 min	150 min
1	0.80 ± 0.07	0.91 ± 0.08	0.94 ± 0.06	0.89 ± 0.03	0.85 ± 0.03	
2a	0.73 ± 0.04	1.10 ± 0.10	1.02 ± 0.09	1.05 ± 0.08	0.93 ± 0.06	
2b	0.76 ± 0.06	1.14 ± 0.09[d)]	1.15 ± 0.09[a, d)]	1.22 ± 0.08[a, d)]	1.06 ± 0.20	
2c	0.79 ± 0.05	0.83 ± 0.05	0.81 ± 0.04	0.81 ± 0.05	0.76 ± 0.04	
3a	0.70 ± 0.01	0.93 ± 0.05	1.13 ± 0.09[d)]	2.53 ± 0.25[c, e)]	2.13 ± 0.18[c, e)]	1.50 ± 0.16[a, d)]
3b	0.79 ± 0.06	0.85 ± 0.07	1.15 ± 0.07[c, e)]	0.89 ± 0.05	0.85 ± 0.07	
3c	0.81 ± 0.05	0.99 ± 0.06	0.91 ± 0.07	0.84 ± 0.05	0.83 ± 0.04	
4a	0.71 ± 0.03	1.01 ± 0.12	0.88 ± 0.04	0.83 ± 0.03	0.75 ± 0.04	
4b	0,75 ± 0.05	0.890 ± 0.04	0.980 ± 0.03[b)]	0.870 ± 0.05	0.790 ± 0.04	
4c	0.80 ± 0.04	2.71 ± 0.21[c, e)]	1.80 ± 0.10[b)]	1.54 ± 0.16[b)]	1.26 ± 0.09[d)]	
Vehicle	0.83 ± 0.05	0.840 ± 0.05	0.860 ± 0.06	0.910 ± 0.91	0.790 ± 0.05	0.82 ± 0.05
Morphine	0.74 ± 0.04	4.88 ± 0.35[c, e)]	2.41 ± 0.25[c, e)]	1.78 ± 0.36[a, d)]	1.27 ± 0.22[d)]	0.90 ± 0.54

The results were expressed as mean ± SEM. Repeated measures of ANOVA was performed for statistical analysis (n = 8 for each group).

[a)] $p < 0.05$, [b)] $p < 0.01$, [c)] $p < 0.001$, when compared with its own baseline value or control group ([d)] $p < 0.05$, [e)] $p < 0.001$).

Experimental

Chemistry

All chemicals and solvents were purchased locally from Aldrich, Fluka, and Merck (Aldrich, Steinheim, Germany, Fluka, Buchs, Switzerland, Merck, Darmstadt, Germany). Melting points were determined by Büchi 530 melting point apparatus (Büchi Labortechnik, Flawil, Switzerland). UV spectra were recorded on a Beckman DU 530 spectrophotometer (Beckman, Palo Alto, CA, USA). The purity of the compounds was checked by high performance liquid chromatography (HPLC) using diode array detector (Agilent 1100, Agilent, Palo Alto, CA, USA). Infrared spectra were recorded on Perkin Elmer 1600 FT-IR spectrophotometer (Perkin Elmer, Beaconsfield, England). ^{1}H-NMR spectra (DMSO-d_6) were run on Bruker AVANC-DPX 400 MHz NMR (Bruker, Rheinstetten, Germany) with TMS internal standard (chemical shift δ in ppm and coupling constant J in Hz). The Mass spectrometry was performed using Agilent 1100 MSD spectrometer in the electrospray mode by Turkish Scientific and Technical Research Council (TUBITAK) laboratory. The IR spectra of all compounds showed the significant bands in the expected regions, O-H and N-H bands around 3462-3155 cm^{-1}. Some significant stretching bands due to C=O, C=N, and C=S for **2a–c** were observed at 1696–1682, 1576–1559, and 1300–1252 cm^{-1}; C=N and C–S–C bands for compounds **3a–c** at 1576–1550 and 668–675 cm^{-1}; C=N and C=S for **4a–c** at 1628–1623 and 1300–1243 cm^{-1}, respectively. In the ^{1}H-NMR spectra of thiosemicarbazide derivatives **2a–c**, N^1-H, N^2-H, and N^4-H protons appeared as singlet at 10.35–10.60, 9.22–9.56, and 7.81–8.37 ppm, respectively.

*Synthesis of thiosemicarbazides **2a–c***

To a solution of hydrazide **1** [23] (0.005 mol) in ethanol (60 mL), isothiocyanate (0.005 mol) was added. The mixture was refluxed for 2 h and ,after cooling, the precipitate formed was recrystallized from ethanol.

*Synthesis of 1,3,4-thiadiazoles **3a–c***

An appropriate thiosemicarbazide (0.001 mol) was dissolved in conc. H_2SO_4 (3 mL). The reaction mixture was stirred for 1 h then poured onto crushed ice. The precipitated solid was filtered, washed with sodium bicarbonate solution and water, respectively, and then recrystallized from ethanol.

*Synthesis of 1,2,4-triazole-3-thiones **4a–c***

A solution of an appropriate thiosemicarbazides (0.01 mol) in 2 N NaOH (25 mL) was refluxed for 6 h, cooled, poured into ice-cold water (60 mL), and neutralized with glacial acetic acid. The precipitate that separated was filtered off, washed with water, and recrystallized from ethanol.

*1-[4-((1-(2-hydroxyethyl)-3,5-dimethyl-1H-pyrazol-4-yl)azo)benzoyl]-4-butyl thiosemicarbazide **2a***

IR (KBr): 3226 (O–H and N–H), 2958 (=C–H), 1682 (C=O), 1559 (C=N), 1418 (N=N), 1252 (C=S) cm^{-1}. ^{1}H-NMR δ (ppm): 0.90 (t, 3H, $-NHCH_2CH_2CH_2\mathit{CH_3}$), 1.29 (m, 2H, $-NHCH_2CH_2\mathit{CH_2}CH_3$), 1.50 (m, 2H, $-NHCH_2\mathit{CH_2}CH_2CH_3$), 2.41 (s, 3H, pyrazole C_5 methyl), 2.61 (s, 3H, pyrazole C_3 methyl), 3.45 (q, 2H, $-NH\mathit{CH_2}CH_2CH_2CH_3$), 3.76 (q, 2H, $-CH_{2CH_2}OH$), 4.11 (t, 2H, $-\mathit{CH_2}CH_2OH$), 4.93 (t, 1H, $-CH_2CH_2\mathit{H}$), 7.81 (d, *J*=8.5 Hz, 2H, *ortho*-protons to azo), 8.08 (m, 3H, *meta*-protons azo and –NHNHCS*NH*-), 9.25 (s, 1H, –NH*NH*CSNH–), 10.39 (s, 1H, –*NH*NHCSNH–). MS (ES) $[M+H]^+$: *m/z* 418.

*1-[4-((1-(2-hydroxyethyl)-3,5-dimethyl-1H-pyrazol-4-yl)azo)benzoyl]-4-cyclohexyl thiosemicarbazide **2b***

IR (KBr): 3318 (O–H and N–H), 2934 (=C–H), 1694 (C=O), 1576 (C=N), 1420 (N=N), 1253 (C=S) cm^{-1}. ^{1}H-NMR δ (ppm): 1.05–1.80 (m, 11H, cyclohexyl protons), 2.41 (s, 3H, pyrazole C_5 methyl), 2.61 (s, 3H, pyrazole C_3 methyl), 3.75 (q, 2H, $-CH_2\mathit{CH_2}OH$), 4.11 (t, 2H, $-\mathit{CH_2}CH_2OH$), 4.93 (t, 1H, $-CH_2CH_2\mathit{OH}$), 7.81 (m, 3H, ortho-protons to azo and –NHNHCS*NH*–), 8.05 (d, 2H, *ortho*-protons azo), 9.22 (s, 1H, –NH*NH*CSNH–), 10.35 (s, 1H, –*NH*NHCSNH–). MS (ES) $[M+H]^+$: *m/z* 444.

*1-[4-((1-(2-hydroxyethyl)-3,5-dimethyl-1H-pyrazol-4-yl)azo)benzoyl]-4-(2-phenethyl) thiosemicarbazide **2c***

IR (KBr): 3238 (O–H and N–H), 2924 (=C–H), 1696 (C=O), 1559 (C=N), 1435 (N=N), 1300(C=S) cm^{-1}. ^{1}H-NMR δ (ppm): 2.61 (s, 3H,

pyrazole C_5 methyl), 2.81 (s, 3H, pyrazole C_3 methyl), 3.20 (t, 2H, $-CH_2CH_2C_6H_5$), 3.90 (q, 2H, $-CH_2CH_2C_6H_5$), 4.01 (q, 2H, $-CH_2CH_2OH$), 4.35 (t, 2H, $-CH_2CH_2OH$), 5.25 (t, 1H, $-CH_2CH_2OH$), 7.43–7.55 (m, 5H, $-CH_2CH_2C_6H_5$), 8.06 (d, *J*= 8.0 Hz, 2H, *ortho*-protons to azo), 8.31 (d, *J*= 8.0 Hz, 2H, *meta*-protons to azo); 8.37 (s, 1H, –NHNHCSNH–), 9.56 (s, 1H, –NHNHCSNH–), 10.60 (s, 1H, –NHNHCSNH–). MS (ES) $[M+H]^+$: *m/z* 466.

2-(Butylamino)-5-[((1-(2-hydroxyethyl)-3,5-dimethylpyrazole-4-yl)azo)phenyl]-1,3,4-thiadiazole ***3a***

IR (KBr): 3177 (O–H and N–H), 2929 (=C–H), 1550 (C=N), 1420 (N=N), 668 (C–S–C) cm^{-1}. ^{1}H-NMR δ (ppm): 0.92 (t, 3H, $-NHCH_2CH_2CH_2CH_3$), 1.33 (m, 2H, $-NHCH_2CH_2CH_2CH_3$), 1.56 (m, 2H, $-NHCH_2CH_2CH_2CH_3$), 2.41 (s, 3H, pyrazole C_5 methyl), 2.61 (s, 3H, pyrazole C_3 methyl), 3.24 (m, 2H, $-NHCH_2CH_2CH_2CH_3$), 3.75 (q, 2H, $-CH_2CH_2OH$), 4.11 (t, 2H, $-CH_2CH_2OH$), 5.25 (t, 1H, $-CH_2CH_2OH$), 7.82 (d, *J*= 8.5 Hz, 2H, *ortho*-protons to azo,), 7.86 (d, *J*= 8.5 Hz 2H, *meta*-protons to azo), 8.10 (s, 1H, –NH–). MS (ES) $[M+H]^+$: *m/z* 400.

2-(Cyclohexylamino)-5-[((1-(2-hydroxyethyl)-3,5-dimethylpyrazole-4-yl)azo)phenyl]-1,3,4-thiadiazole ***3b***

IR (KBr): 3248-3192 (O–H and N–H), 3065 (=C–H), 1559 (C=N), 1418 (N=N), 675 (C–S–C) cm^{-1}. ^{1}H-NMR δ (ppm): 1.30–2.03 (m, 11H, cyclohexyl protons), 2.41 (s, 3H, pyrazole C_5 methyl), 2.61 (s, 3H, pyrazole C_3 methyl), 3.75 (q, 2H, $-CH_2CH_2OH$), 4.11 (t, 2H, $-CH_2CH_2OH$), 4.32 (t, 1H, $-CH_2CH_2OH$), 7.81–7.95 (m, 4H, ArH), 8.70 (s, 1H, $-NH$-C_6H_{11}). MS (ES) $[M+H]^+$: *m/z* 426.

2-[(2-Phenylethyl)amino]-5-[((1-(2-hydroxyethyl)-3,5-dimethylpyrazole-4-yl)azo)phenyl]-1,3,4-thiadiazole ***3c***

IR (KBr): 3420 (O–H and N–H), 2925 (=C–H), 1576 (C=N), 1419 (N=N), 668 (C–S–C) cm^{-1}. ^{1}H-NMR δ (ppm): 2.65 (s, 3H, pyrazole C_5 methyl), 2.91 (s, 3H, pyrazole C_3 methyl), 3.20 (t, 2H, $-CH_2CH_2C_6H_5$), 3.88 (q, 2H, $-CH_2CH_2OH$), 3.99 (q, 2H, $-CH_2CH_2C_6H_5$), 4.30 (t, 2H, $-CH_2CH_2OH$), 4.52 (t, 1H, $-CH_2CH_2OH$), 7.46-8.20 (m, 9H, ArH), 10.70 (s, 1H, $-NHCH_2CH_2C_6H_5$). MS (ES) $[M+H]^+$: *m/z* 448.

4-[((1-(2-Hydroxyethyl)-3,5-dimethylpyrazole-4-yl)-azo)phenyl]-4-buthyl-2,4-dihydro-3H-1,2,4-triazole-3-thione ***4a***

IR (KBr): 3423–3108 (O–H and N–H), 2919 (=C–H), 1623 (C=N), 1408 (N=N), 1243 (C=S) cm^{-1}. ^{1}H-NMR δ (ppm): 0.78 (t, 3H, $-NHCH_2CH_2CH_2CH_3$), 1.18 (m, 2H, $-NHCH_2CH_2CH_2CH_3$), 1.55 (m, 2H, $-NCH_2CH_2CH_2CH_3$), 2.42 (s, 3H, pyrazole C_5 methyl), 2.62 (s, 3H, pyrazole C_3 methyl), 3.75 (m, 4H, $-NHCH_2CH_2CH_2CH_3$ and $-CH_2CH_2OH$), 4.11 (t, 2H, $-CH_2CH_2OH$), 4.95 (t, 1H, $-CH_2CH_2OH$), 7.83 (d, *J*= 8.5 Hz, 2H, *ortho*-protons to azo), 7.88 (d, *J*= 8.5 Hz, 2H, *meta*-protons to azo), 13.99 (s, 1H, NH proton of triazole). MS (ES) $[M+H]^+$: *m/z* 400.

4-[((1-(2-Hydroxyethyl)-3,5-dimethylpyrazole-4-yl)azo)phenyl]-4-cyclohexyl-2,4-dihydro-3H-1,2,4-triazole-3-thione ***4b***

IR (KBr): 3155–3108 (O–H and N–H), 2927 (=C–H), 1628 (C=N), 1413 (N=N), 1243 (C=S) cm^{-1}. ^{1}H-NMR δ (ppm): 1.19–2.68 (m, 17H, cyclohexyl protons, pyrazole C_5 methyl and pyrazole C_3 methyl), 3.75 (q, 2H, $-CH_2CH_2OH$), 4.12 (t, 2H, $-CH_2CH_2OH$), 4.94 (t, 1H, $-CH_2CH_2OH$), 7.69 (d, *J*= 8.3 Hz, 2H, *ortho*-protons to azo), 7.88 (d, *J*= 8.2 Hz 2H, *meta*-protons to azo), 13.99 (s, 1H, NH proton of triazole). MS (ES) $[M+H]^+$: *m/z* 426.

4-[((1-(2-Hydroxyethyl)-3,5-dimethylpyrazole-4-yl)azo)phenyl]-4-(2-phenethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione ***4c***

IR (KBr): 3462 (O–H and N–H), 2932 (=C–H), 1625 (C=N), 1419 (N=N), 1245 (C=S) cm^{-1}. ^{1}H-NMR δ (ppm): 2.42 (s, 3H, pyrazole C_5 methyl), 2.62 (s, 3H, pyrazole C_3 methyl), 2.99 (t, 2H, $-CH_2CH_2C_6H_5$), 3.76 (q, 2H, $-CH_2CH_2OH$), 4.12 (t, 2H, $-CH_2CH_2C_6H_5$), 4.28 (t, 2H, $-CH_2CH_2OH$), 4.94 (t, 1H, $-CH_2CH_2OH$), 7.00-7.23 (m, 5H, $-CH_2CH_2C_6H_5$), 7.58 (d, *J*= 8.4 Hz, 2H, *meta*-protons to azo), 7.81 (d, *J*= 8.4 Hz, 2H, *ortho*-protons to azo), 14.01 (s, 1H, NH proton of triazole). MS (ES) $[M+H]^+$: *m/z* 447.

Pharmacology

All experimental protocols were approved by the Marmara University School of Medicine Animal Care and Use Committee. Adult Balb/C male and female mice (25-30 g) were used in the study. They were housed in a quiet, temperature- (20 ± 2°C) and humidity- (60 ± 3%) controlled room, where a 12/12 h light-dark cycle was maintained (07:00–19:00 light). The mice were fed standard lab chow and tap water *ad lib* during the study. The thermal techniques (tail-immersion [24] and hot-plate [25]) were used to evaluate both basal nociceptive threshold and the analgesic effect of the compounds **2a–c**, **3a–c**, and **4a–c**. All substances were suspended in 0.5% methyl cellulose (MC) and administered at a dose of 100 mg/kg by intraperitoneal injection (*i.p.*) in a volume of 0.1 mL/10 g. Control animals received the vehicle which is 0.5% MC. For the reference analgesic drug morphine, HCl was suspended in the same vehicle and given at a dose of 5 mg/kg. Bederson-modified neurological examination [26] was also conducted with mice to verify that the dose used did not produce neurological side effects. Briefly, in the hot-plate test, the licking of the hind paw or jumping was measured as hot-plate latency at 55°C and in the tail-immersion test, the mice tails were immersed in warm water (55°C) which provokes an abrupt movement of the tail and sometimes the recoiling of the whole body. Analgesia is defined as the increase in the baseline latency. Mice were either injected with vehicle (control group), morphine hydrochloride (reference analgesic for both tests) 5 mg/kg *i.p.*, or compounds 100 mg/kg *i.p.* Test duration was 120 min after the injection of the drug. Each animal served as its own control and 20 s (for hot-plate), 10 s (for tail-immersion) were used as a cut-off latency to avoid tissue damage. Test duration was 120 min for substances. Only the **4a** group was observed to 150 min since the activity was still continuing.

Statistics

Statistical analysis was carried out using GraphPad Prism 3.0 (GraphPad Software, San Diego, CA, USA). All data were expressed as means ± SEM. Groups of data for analgesia tests were compared with an analysis of variance (ANOVA) of repeated measures, followed by Tukey's multiple comparison tests. Neurological examination scores were compared with ANOVA test followed by Tukey's post-hoc test. Values of $p < 0.05$ were regarded as significant.

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