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Synthesis and Pharmacological Screening for Muscle Relaxant, Anticonvulsant, and Sedative Activities of Certain Organic Compounds Produced by Michael Addition

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Michael addition of certain nucleophiles on α, β-unsaturated ketones **1** led to the formation of adducts **2-7** as well as the reaction of arylidene derivatives with secondary amines afforded the amino compounds **9** and **11**. Also, dialkylmalonates were treated with α-cyano cinnamide to afford **13**. On the other hand, double Michael cycloaddition of ethylcyanoacetate or tetrachlorophthalic anhydride to the suitable divinylketone were synthesized to produce **15-17**. Selected compounds **(13** and **6)** were screened for muscle relaxant, anticonvulsant, and sedative activities using established pharmacological models. Their activities were compared with that of phenobarbital sodium taken as standard. Compound **6** was the most potent muscle relaxant while compounds **13a** and **13c** offered the highest anticonvulsant activity. Meanwhile compound **13c** showed the highest potentiation of phenobarbital induced sleep in mice.

Key words: Michael addition, 2-Pyridone, Muscle relaxant, Anticonvulsant, Sedative activities

INTRODUCTION

Although literature is full of Michael adducts which belong to a variety of chemical structures (Sykes, 1985; Carrol, 1988; Volhard, 1999; March, 2000), few of them have been screened as potential biologically active agents (Reddy *et al.*, 1992; Osman *et al.*, 1996). Certain spiro compounds related to substituted barbiturates were found to exert psychotropic (Allen *et al.*, 1972), hypnotic (Meyer *et al.*, 1976), antineoplastic (De and Pal, 1977) and analgesic (Bose *et al.*, 1985; Abou-El-Enein and Azeouny, 1988) activities. Moreover the 2-pyridones are an important class of compounds that attracted the attention of medicinal chemists as chemotherapeutic agents. The sedative (Collins *et al.*, 2002) anticonvulsant (Goehring *et al.*, 1990), antiparkinson (Sergio *et al.*, 1992), and antiatherosclerotic (James *et al.*, 1995) activities of 2-pyridone derivatives are well documented. Accordingly, the present study focused on the application of Michael reaction to produce novel heterocyclic compounds. The study also screened synthesized compounds for their potential muscle relaxant, anticonvulsant, and sedative activities.

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MATERIALS AND METHODS

Chemistry

The melting points were determined on a Griffin apparatus and were uncorrected. Elemental analyses were performed at the Faculty of Pharmacy, King Saud University. IR spectra were recorded on a Perkin Elmer FT spectrophotometer 1000. NMR spectra were recorded on a Jeol 200 MHz with tetramethylsilane as internal standard (DMSO-d_6). Chemical shifts are given in ppm and coupling constants (J) are given in Hz. Mass spectra were run on a Hewlett Packard HP 5988A (EI). The progress of the reactions was monitored using TLC sheets precoated with UV Fluorescent silica gel Merck 60 F 254. The reactions were visualized using a UV lamp.

1,3,3-Trisubstituted propan-1-ones (2a-c and e), (3c and e), (4b and e)

Method A : for 2a-c,e & 3c,e

A mixture of equimolar (0.01 mol) chemicals of either thiobarbituric acid or dimethylcyclohexanedione in ethanol (15 mL), the appropriate α,β-unsaturated ketone **(1a-c,e)**

in ethanol (15 mL) and 3 drops of triethylamine was refluxed for 3 h. After cooling, the separated solid was filtered and crystallized from ethanol (Table I, II).

Method B: for 4b,e

To a 0.01 mol solution of α,β-unsaturated ketone **(1b,e)** in dry benzene (20 mL), 5-bromoindole (0.01 mol) was added and the mixture was refluxed for 24 h. The solid that was separated from the liquid during the refluxing was filtered and crystallized from ethanol (Table I, II).

2,4-Disubstituted-2,3-dihydro-1*H*-naphtho[b][1,4] diazepines (5b-e)

A solution of 2,3-diaminonaphthalene (0.01 mol) and 1,3-diaryl-2-propenone (0.01 mol) in absolute ethanol (15 mL) and acetic acid (1 mL) was refluxed for 4 h. After being neutralized with ammonia and cooled down to 0°C, the mixture was allowed to stand overnight. The resulting precipitate was filtered and recrystallized from ethanol (Table I, II).

3-Cyano-4-(3',4',5'-trimethoxyphenyl)-6-(3'-methyl phenyl)-1,2,3,4-tetrahydro-2-pyridone (6)

An equimolar mixture of trimethoxy benzal methylacetophenone **1e** and cyanoacetamide (0.01 mol) in an ethanolic solution of sodium ethoxide (prepared from 0.4 g of metallic sodium and 20 mL of absolute ethanol) was stirred for 24 h at ambient temperature and then heated at 50-60°C for 1 h. The reaction mixture was concentrated and poured onto ice-water (50 mL) containing hydrochloric acid (10 mL). The solid obtained from the separation was filtered and crystallized from benzene/ethanol (Table I, II).

2,6-Bis(3'-methylphenyl)4-(3',4',5'-trimethoxyphenyl), 1,4-dihydropyridine (7)

A mixture of trimethoxy benzal methyl acetophenone **(1e)** (0.01 mol), 3-methyl acetophenone (0.02 mol) and ammonium acetate (2 g) in glacial acetic acid (10 mL) was refluxed for 3 h. The reaction mixture was concentrated, cooled, and poured onto ice water. The separated solid was filtered and crystallized from ethanol (Table I, II).

5-[(*N,N*-Disubstituted)-aminobenzyl]-thiobarbituric acid (9a-c) and 2-[(*N,N*-disubstit)-aminobenzyl]5,5-dimethyl-2,3-cyclohexanedione (11a-c)

General procedure

A mixture of the appropriate 5-arylidenethiobarbituric acid **(8)** or 2-arylidene dimethylcyclohexanedione **(10)** (0.001 mol) and the suitable secondary amine (0.002 mol) in absolute ethanol (50 mL) was stirred at room temperature for 24 h. The solid obtained was filtered, washed with water and crystallized from aqueous ethanol (Table I, II).

4-Aryl-3-cyano-5-methoxy [or ethoxy]carbonyl-2,6-piperidinediones (13a-c)

A mixture of the appropriate α-cyanocinnamide **1** (0.01 mol) and dimethyl (or diethyl) malonate (0.01 mol) in an ethanolic solution of sodium ethoxide (prepared from 0.4 g of metallic sodium and 10 mL of absolute ethanol) was stirred for 12 h at room temperature. The reaction mixture was then heated at 50-60°C for 1 h, concentrated and poured onto ice cold water (50 mL) containing hydrochloric acid (10 mL). The solid separated from the liquid was filtered and crystallized from benzene/ethanol (Table I, II).

3,5-Bis(3',4',5'-trimethoxyphenyl)-4-cyano-4-ethoxycarbonylcyclohexanone (15)

An ethanolic solution of sodium ethoxide (prepared from 0.15 g of metallic sodium and 5 mL of absolute ethanol) was added to a solution of the divinyl ketone **14** (0.44 mol) and ethyl cyanoacetate (0.52 mol) in absolute ethanol (10 mL). The solution was refluxed for 3 h, then concentrated, cooled, and poured onto ice cold water. The separated solid was filtered and crystallized from aqueous ethanol (Table I, II).

1-Amino-6,10-bis(3',4',5'-trimethoxyphenyl)-2,3-diazaspiro (4.5)-1-decane-4,8-dione-8-hydrazone (16)

An equimolar solution of ester **15** and hydrazine hydrate (95%) (0.01 mol of each) in absolute ethanol (10 mL) was refluxed for 12 h. The reaction mixture was concentrated, cooled, and poured onto ice cold water. The separated precipitate was filtered and crystallized from benzene/ethanol (Table I, II).

4',5',6',7'-Tetrachloro-2,6-bis(3',4',50'-trimethoxyphenyl)-4*H*-spiro(cyclohexan-1,2'-indane)-1',3',4-triones (17)

A solution of tetracloro-1,3-indandione (0.005 mol) in hot 95% Ethanol (10 mL) was added to a solution of divinyl ketone **1e** (0.005 mol) in dioxane (10 mL) and 3 drops of triethylamine. The resulting solution was heated under reflux for 7 h, cooled, and filtered. The filtrate was concentrated and poured onto icecold water, where upon the solid product produced was filtered and crystallized from ethanol (Table I, II)

Pharmacological screening procedures

Chemicals

Phenobarbital sodium and strychnine (98%) were purchased from Sigma Aldrich Chemical Co (St. Louis, USA).

Table I. Physical, analytical and IR spectral data of Synthesized compounds

Comp. No.	m.p. (°C)	Yield %	Mol. Form. (Mol. Wt.)	Elememtal Analysis % Calcd./Found			IR KBr (cm^{-1})
				C	H	N	
2a	170	70	$C_{20}H_{17}BrN_2O_3S$ (445)	53.93 53.90	3.82 3.80	6.29 6.20	3450-3420, 3100-3062 (2 NH), 2900-2700 (CH aliph) 1700, 1670, 1654 (3 C = O), 1368-1194 (C = S).
2b	140	73	$C_{17}H_{13}ClN_2O_3S_2$ (392.5)	51.97 51.95	3.31 3.30	7.13 7.00	3450-3423, 3100-3064 (2 NH), 2900-2750 (CH aliph) 1700, 1670, 1644 (3 C = O), 1315-1212 (C = S).
2c	218	70	$C_{27}H_{19}ClN_2O_3S$ (486.5)	66.59 66.60	3.90 3.90	5.75 5.70	3460-3435, 3100-3006 (2 NH), 2920-2810 (CH aliph) 1690, 1681, 1658 (3 C = O), 1390-1180 (C = S).
2e	190	68	$C_{23}H_{24}N_2O_6S$ (456)	6052 60.52	5.26 5.25	6.14 6.10	3450-3370, 3100-3080 (2NH), 2920-2830 (CH aliph) 1700, 1673, 1653 (3 C = O), 1361-1181 (C = S).
3c	139	65	$C_{31}H_{27}ClO_3$ (482.5)	77.09 77.00	5.59 5.60	- -	2920-2800 (CH aliph) 1680, 1670, 1650 (3 C = O).
3e	178	67	$C_{27}H_{32}O_6$ (452)	71.68 71.70	7.07 7.00	- -	2940-2830 (CH aliph) 1680, 1670, 1650 (3 C = O).
4b	125	60	$C_{21}H_{15}BrClNOS$ (444.5)	56.69 56.70	3.37 3.30	3.14 3.10	3330-3270 (NH), 1650 (C = O).
4e	110	68	$C_{27}H_{26}BrNO_4$ (508)	63.77 63.80	5.11 5.10	2.75 2.80	3336-3271 (NH), 1660 (C = O).
5b	140	73	$C_{23}H_{17}ClN_2S$ (388.5)	71.04 71.00	4.37 4.40	7.20 7.20	3420-3330 (NH) 1650 (C = N).
5c	110	70	$C_{33}H_{23}ClN_2$ (482.5)	82.07 82.00	4.76 4.60	5.80 5.70	3420-3330 (NH), 1610 (C = N).
5d	170	75	$C_{33}H_{24}N_2$ (448)	88.39 88.40	5.35 5.30	6.25 6.20	3412-3310 (NH), 1616 (C = N).
5e	140	71	$C_{29}H_{28}N_2O_3$ (452)	76.99 76.90	6.19 6.20	6.19 6.20	3420-3327 (NH), 1658 (C = N).
6	285	75	$C_{22}H_{22}N_2O_4$ (378)	69.84 69.80	5.82 5.80	7.40 7.40	3550-3408 (NH), 2242 (C = N), 1713 (C = O).
7	189	60	$C_{28}H_{28}NO_3$ (426)	78.87 78.90	6.57 6.50	3.28 3.30	3500-3415 (NH), 1591-1570 (C = C aromatic).
9a	170	75	$C_{18}H_{23}Cl_2N_3O_5S$ (463)	46.65 46.6	4.96 5.0	9.07 9.00	3440-3400, 3180-3070 (2NH); 1684, 1630 (2C = O), 1390-1185 (C = S).
9b	206	76	$C_{18}H_{23}N_3O_5S$ (393)	54.96 54.90	5.85 5.80	10.68 10.60	3425-3400, 3187-3066 (2NH); 1726, 1640 (2C = O); 1366- 1167 (C = S).
9c	222	75	$C_{19}H_{25}N_3O_5S$ (407)	56.01 56.00	6.14 6.00	10.31 10.30	3402-3144, 3186-3067 (2NH), 1630, 1602 (2C = O), 1365-1190 (C = S).
11a	213	72	$C_{22}H_{31}Cl_2NO_5N$ (459)	57.51 57.50	6.75 6.70	3.05 3.00	1667, 1625 (2C = O).
11b	206	68	$C_{22}H_{31}NO_5N$ (389)	67.86 67.80	7.96 8.00	3.59 3.50	2927-2800 (CH aliph), 1723, 1674 (2C = O).
11c	200	70	$C_{23}H_{33}NO_5N$ (403)	68.48 68.50	8.18 8.10	3.47 3.40	2961-2824 (CH aliph), 1711, 1674 (2C = O).
13a	210	65	$C_{17}H_{18}N_2O_7$ (362)	56.35 56.30	4.97 4.90	7.73 7.70	3418-3400 (NH); 1696, 1610, 1601 (3C = O).
13b	285	64	$C_{18}H_{20}N_7O_7$ (376)	57.44 57.40	5.31 5.030	7.44 7.40	3433-3410 (NH), 1690, 1620, 1604 (3C = O).
13c	180	65	$C_{17}H_{18}N_2O_6$ (352)	57.95 57.90	5.11 5.10	7.95 7.90	3440-3415 (NH); 1699, 1658, 1720 (3C = O).
15	106	60	$C_{28}H_{33}NO_9$ (527)	63.75 63.70	6.26 6.20	2.65 2.70	2200 (C =N), 1727, 1669 (2C = O).
16	190	63	$C_{26}H_{33}N_5O_7$ (527)	59.20 59.20	6.26 6.20	13.28 13.20	3415-3300 (NH_2, NH) 1728 (C = O), 1620 (C = N).
17	110	60	$C_{32}H_{28}Cl_4O_9$ (556)	69.06 69.00	5.03 5.00	- -	1740, 1720, 1700 (C = O).

Table II. ^{1}H-NMR, ^{13}CNMR and EIMS spectral data of the synthesized novel compounds

Compound No.	Spectral Data
2a	^{1}H-NMR (DMSO-d_6): δ 1.76 (d, 2H, CH_2), 3.06 (q, 1H, CH), 4.30 (m, 1H, C_5H), 7.107.50 (m, 8H aromatic protons), 11.6 (br.s, 2H, 2NH, D_2O exchangeable).
2b	^{1}H-NMR (DMSO-d_6): δ 1.79 (d, 2H, CH_2), 3.07 (q, 1H, CH), 4.40 (m, 1H, C_5H), 7.20 7.60 (m, 7H aromatic and thiophene protons), 11.2 (br.s, 2H, 2NH, D_2O exchangeable).
2c	^{1}H-NMR (DMSO-d_6): δ 2.11 (d, 2H, CH_2), 3.07 (q, 1H, CH), 4.70 (m, 1H, C_5H), 7.13 8.59 (m, 13H aromatic protons), 5.63 (br.s, 2H, 2NH, D_2O exchangeable).
2e	^{1}H-NMR (DMSO-d_6): δ 1.79 (d, 2H, CH_2),2.50 (s, 3H, CH_3) 3.28 (q, 1H, CH), 4.24 (m, 1H, C_5H), 5.06 (s, 9H, $3CH_3$), 7.20 7.60 (m, 6H aromatic protons), 11.01 (br.s, 2H, 2NH, D_2O exchangeable).
3c	^{1}H-NMR (DMSO-d_6): δ 1.90 (d, 2H, CH_2),2.50 (s, 6H, $2CH_3$), 3.30 (q, 1H, CH), 3.38 (s, 4H, $2CH_2$) 3.69 (m, 1H, CH), 7.60 8.80 (m, 13H aromatic protons).
3e	^{1}H-NMR (DMSO-d_6): δ 1.77 (d, 2H, CH_2),2.51 (s, 3H, CH_3) 3.31 (q, 1H, CH), 4.00 (m, 1H, CH), 4.90 (s, 9H, $3CH_2$), 7.11 7.46 (m, 6H aromatic protons). C^{13}-NMR: 21.19, 21.27, 39.77, 40.60, 55.88, 56.33, 75.56, 105.85, 106.40, 125.06, 128.66, 128.72, 137.28, 137.59, 138.46, 147.64, 203.65, 205.76.
4b	^{1}H-NMR (DMSO-d_6): δ 1.74 (d, 2H, CH_2), 3.70 (q, 1H, CH), 7.60 8.80 (m, 11H, aromatic protons), 12.00(br, 1H, NH, D_2O exchangeable).
4e	^{1}H-NMR (DMSO-d_6): δ 1.75 (d, 2H, CH_2), 3.63 (q, 1H, CH), 7.80 8.70 (m, 10H, aromatic protons), 12.00 (br, 1H, NH, D_2O exchangeable).
5b	^{1}H-NMR (DMSO-d_6): δ 1.18 (t, 2H, CH_2), 6.05-6.10 (m, 1H, CH), 7.67-8.27 (m, 13H, aromatic protons), 8.85-8.86(d, 1H, NH), (D_2O exchangeable).
5c	^{1}H-NMR (DMSO-d_6): δ 1.08 (t, 2H, CH_2), 6.06-6.10 (m, 1H, CH), 7.35 8.02 (m, 19 H aromatic protons), 8.63 8.71 (d, 1H, NH, D_2O exchangeable). C^{13}-NMR, δ40.35, 123.55, 124.21, 127.70 127.78, 127.94, 128.12, 128.76, 129.33, 129.56, 129.92, 130.24, 130.54, 130.73, 131.13, 131.28, 131.35, 136.72, 138.83, 141.56.
5d	^{1}H-NMR (DMDO-d_6): poorly absorbed δ 1.00 (t, 2H, CH_2), 6.05 -6.40 (m, 1H, CH), 7.35-8.02 (m, 20H aromatic protons), 8.63-8.71 (d,1H, NH, D_2O exchangeable).
5e	^{1}H-NMR (DMSO-d_6): δ 1.10 (t, 2H, CH_2), 2.40 (s, 3H, CH_3), 3.76 (s, 9H, $3CH_3$), 6.05-6.40(m, 1H, CH), 7.40-8.01 (m, 12H, aromatic protons), 8.80-8.82 (d, 1H, NH, D_2O exchangeable).
6	^{1}H-NMR (DMSO-d_6): δ 2.50 (s, 3H, CH_3), 3.76 (s, 9H, $3CH_3$) d7.7 (d, 1H, C_3H), 7.30 7.80 (m, 6H aromatic protons), 8.14 (s, 1H, NH, D_2O exchangeable).
7	^{1}H-NMR (DMSO-d_6): δ 7.50-8.40 (m, 11H, aromatic C_4H), 8.20 (s, 1H, NH, D_2O exchangeable).
9a	^{1}H-NMR (DMSO-d_6): δ 2.50-3.00 (m, 8H, $4CH_2$), 3.50 (s, 9H, $3OCH_3$) 5.09 (s, 1H, C_5H), 7.00 (s, 2H aromatic protons), 10.90 (s 2H, 2NH, D_2O exchangeable).
9b	^{1}H-NMR (DMSO-d_6): δ 1.88-1.93 (s, 4H, $2CH_2$ of pyrrolidine ring), 2.50 2.51 (m, 4H, CH_2-N-CH_2) 3.37 (s, 9H, $3OCH_3$), 5.08 (s, 1H, C_5H), 7.00-7.01 (s, 2H aromatic protons), 10.92 (br.s, 2H, 2NH, D_2O exchangeable). C^{13}-NMR 23.83, 40.04, 53.80, 60.50, 67.27, 105.67, 135.07, 137.50, 153.13, 162.56, 174.77 EIMS m/z 393, M^+ (0.52%). .
9c	^{1}H-NMR (DMSO-d_6): δ 1.51-1.74 (m, 6H, $3CH_2$ of pyrrolidine ring), 2.49 -2.51 (m, 4H, CH_2-N-CH_2) 3.36 (s, 9H, $3OCH_3$), 5.15 (s, 1H, C_5H), 7.00 (s, 2H, aromatic protons), poorly absorbed at 6.30 (s, 2H, 2NH). C^{13}-NMR: 22.72, 39.22, 40.61, 51.27, 66.89, 106.21, 133.53, 137.94, 153.33, 162.78, 174.80.
11a	^{1}H-NMR (DMSO-d_6): d1.03 (s, 6H, $2CH_3$), 2.50-3.00 (m, 8H, $4CH_2$), 3.30(s, 9H, $3CH_3$), 5.09 (s, 1H, CH) 3.50 (s, 4H, $2CH_2$), 7.00 (s, 2H aromatic protons).
11b	^{1}H-NMR (DMSO-d_6): δ 2.09-2.25 (s, 4H, $2CH_2$ of pyrrolidine ring), 2.50 -2.51 (m, 4H, CH_2-N-CH_2) 3.39 (s, 9H, $3OCH_3$), 3.59 (s, 4H, $2CH_2$), 3.68 (s, 6H, 2CH3), 4.48 (s, 1H, CH), 6.41 (s, 2H, aromatic protons). C^{13}-NMR: 26.91, 29.25, 32.43, 39.51, 40.90, 50.59, 56.31, 105.93, 114.80, 152.89, 163.59, 196.73.
11c	^{1}H-NMR (DMSO-d_6): δ 1.50-1.74 (m, 6H, $3CH_2$ of piperidine ring), 2.49 -2.50 (m, 4H, CH_2-N-CH_2) 3.34 (s, 9H, $3OCH_3$), 3.59 (s, 4H, $2CH_2$), 3.68 (s, 6H, $2CH_3$), 5.15 (s, 1H, CH), 6.92 (s, 2H aromatic protons). C^{13}-NMR: 26.92, 29.25, 32.43, 39.79, 40.63, 50.59, 56.31, 105.94, 114.80 140.45, 152.89, 163.59, 196.73 EIMS m/z 403 M^+ -3 (100%).
13a	^{1}H-NMR (DMSO-d_6): δ 0.67 (t, 3H, CH_3), 3.62-4.00 (m, 5H, CH_2, 3CH), 3.90 (s, 6H, $2OCH_3$), 4.90 (br.s, 1H, NH, D_2O exchangeable), 7.10 -7.60 (3H, aromatic protons).
13b	^{1}H-NMR (DMSO-d_6): δ 3.70-4.00 (m, 6H, CH_3, 3CH), 3.82 (s, 9H, $3OCH_3$), 4.63 (s, 1H, NH, D_2O exchangeable), 7.20 -7.63 (s, 2H, aromatic protons).
13c	^{1}H-NMR (DMSO-d_6): δ 0.82 (t, 3H, CH_3), 3.52-4.02 (m, 5H, CH_2, 3CH), 3.80 (s, 9H, $3OCH_3$), 4.76 (s, 1H, NH, D_2O exchangeable), 7.12 -7.50 (3 aromatic protons).
15	^{1}H-NMR (DMSO-d_6): δ 3.30 (t, 5H, 2CH, CH_3 of CH_2CH_3), 2.4 (q, 2H, CH_2-CH_3), 2.50 (d, 4H, $2CH_2$). C^{13}-NMR: 39.21, 39.50, 39.77, 56.62, 60.70, 106.65, 125.77, 130.83, 140.14, 143.46, 153.69, 188.78, 188.81. EIMS m/z 527; M^+ (0.11%).
16	^{1}H-NMR (DMSO-d_6): δ 2.49-2.51 (t, 2H, 2CH), 3.40-3.72 (d, 4 H, $2CH_2$), 3.70 (s, 9H, CH_3), 5.20(s, 1H, NH, D_2O exchangeable), 7.10- 7.70 (m, 4H, aromatic protons).10.50 (b, 4H, $2NH_2$ D_2O exchangeable). EIMS m/z 527, M^+ (0%) 428 (100%).
17	^{1}H-NMR (DMSO-d_6): δ 3.7 (s, 9H, CH_3) δ 2.50-2.70 (d, 4H, $2CH_2$), 3.00-3.30 (t, 2H, 2CH), 7.20-8.20 (m, 4H aromatic protons).

Animals

Adult male Swiss Albino mice, 20-25 g, were obtained from the animal house facility at King Saud University. The animals were housed in cages with 12/12 h light/dark cycle at 21±2°C. The animals were given Purina rat chow and water *ad libitum*. They were kept under observation for one week prior to the start of treatment.

All animal experiments were carried out in accordance with King Saud University Ethical Committee Acts.

Muscle relaxant activity (traction test)

The forepaws of the mouse were placed on a small twisted wire rigidly supported above a bench top. Normal mice grasped the wire with their forepaws and when allowed to hang free, placed at least one hind foot on the wire within 5 seconds. The inability to put up at least one hind foot constituted failure to the traction test (Villar *et al.*, 1992). The test was conducted in groups of ten previously screened animals, 15 minutes after the injection of either saline phenobarbital (90 mg/kg, i.p.) or the test compounds (**13a-13c** and **6**) (50 mg/kg, i.p.).

Anticonvulsant activity (strychnine-induced seizure test)

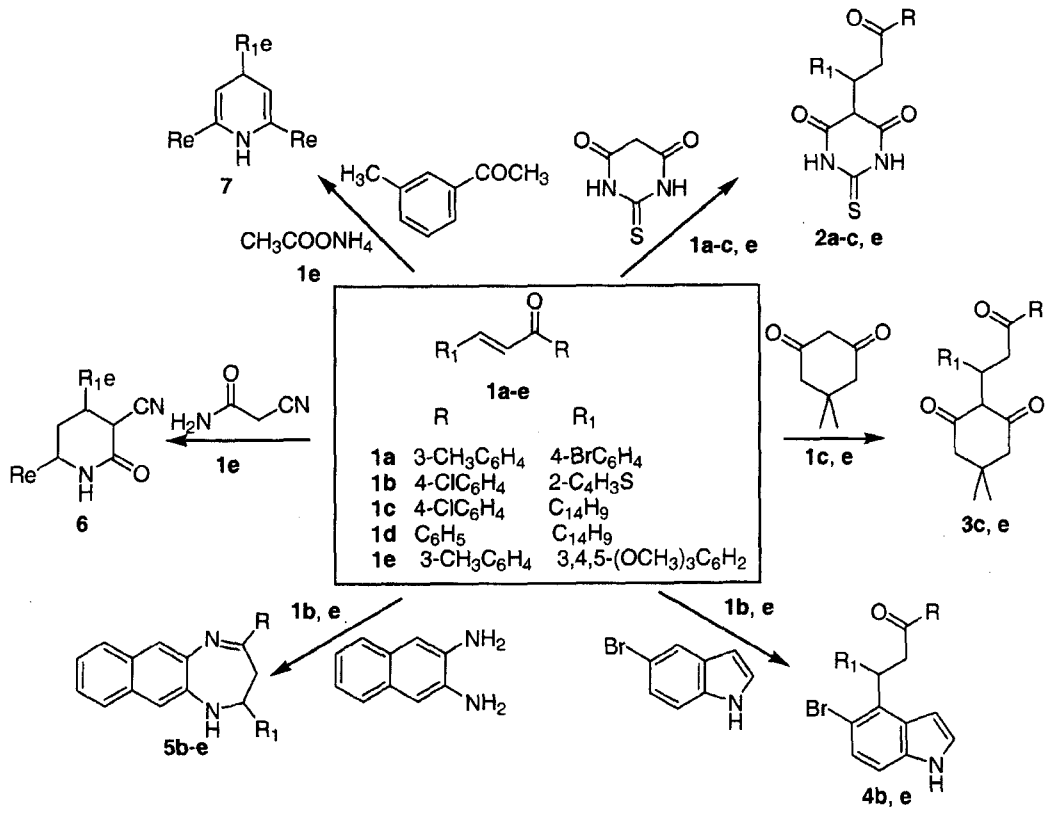
The mice were divided into groups of ten. Test compounds (**13a-13c** and **6**), phenobarbital sodium, and normal saline were injected i.p. into separate groups at the doses of 50 mg/kg, 200 mg/kg, and 10 mL/kg, respectively. After 30 and 60 minutes, the animals were injected with strychnine (0.2 mg/kg i.p.). The onset of seizures was used as the endpoint. The time taken before the onset of convulsions, the percentage of seizure protection, and mortality percentage were recorded.

Sedative/Hypnotic activity (potentiation of phenobarbital sodium-induced sleep)

The mice were divided into five groups of eleven animals each. One group received 90 mg/kg phenobarbital sodium i.p.. Each of the remaining groups received 50 mg/kg i.p. of one of the test compounds (**13a-13c** and **6**). One hour after the administration of the test compound, each animal was injected with 90 mg/kg i.p. phenobarbital sodium. The onset and duration of sleep were noted by recording the time interval elapsed between the loss and regaining of righting reflex as previously explained by Vogel and Vogel (1997).

Statistical analysis

The data were expressed as mean±SEM and drug potency compared to control was tested using the Student *t*-test ($p<0.05$). The comparison among the different test



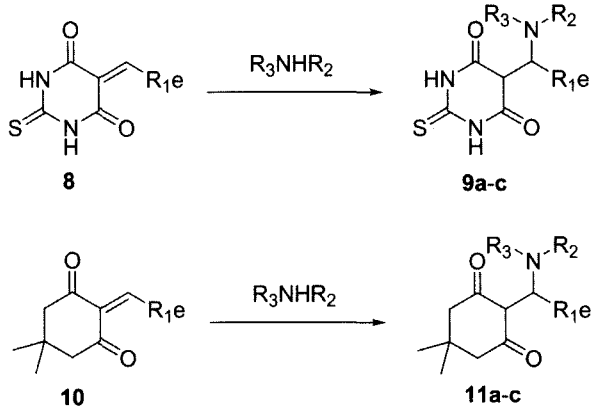
Scheme 1. Preparation of compounds **2**~**7**

groups (compounds **13a-13c** and **6**) was done using one way analysis of variance (ANOVA) followed by the multiple comparison tests of Tukey-Kramer ($p<0.05$).

RESULTS AND DISCUSSION

Chemistry

Michael addition of thiobarbituric acid, 5,5-dimethyl cyclohexane 1,3 diones or 5-bromo indole as nucleophilic compounds to the α,β-unsaturated ketones **1a-e** which were prepared according to a previously reported procedure (Braulio *et al.*, 1997) led to 1,3,3-trisubstituted propan-1-ones (**2a-c,e; 3c,e; 4b,e**). In addition, the refluxing of these unsaturated ketones with 2,3-diamino naphthalene was done in absolute ethanol and acetic acid for 4 h to afford the diazepine derivatives **5b-e**. Furthermore, the reaction of 1-(3-methyl phenyl)-3-(3,4,5-trimethoxy phenyl) prop-2-en-1-one with either cyanoacetamide or 3-methyl acetophenone in ethanolic solution of sodium ethoxide or in presence of acetic acid and ammonium acetate yielded 3-cyano 4-trimethoxyphenyl-6-methylphenyl-1,2,3,4-tetrahydro-2-pyridone (**6**), 2,6-dimethylphenyl,-4-trimethoxy phenyl 1,4-dihydro pyridine **(7)** respectively (Scheme 1). On the other hand, trimethoxy benzylidene derivatives **8** and **10** were stirred in ethanol with a number of secondary amines to afford the adducts **9a-c** and **11a-c**, respectively (Scheme 2). The synthesis of 2,6-piperidindione derivatives **13** was achieved by the Michael addition of dialkyl malonates to benzylidene cyanoacetamide derivative **12** which were obtained by reaction of cyanoacetamide with certain aromatic aldehydes according to the reported procedure (Reddy and Varma, 1997) (Scheme 3). In addition, the cyano ethoxy carbonyl cyclohexanone **15** was prepared by the double Michael cycloaddition of ethyl cyanoacetate to the divinyl ketone **14**. Also the spiro compound **16** was obtained by refluxing the cyanoester derivative **15** with hydrazine hydrate for 12 h. Finally, the ethanolic solution of divinyl ketone **14** was heated for 7 h under reflux with a solution of tetrachloro phthalic anhydride in dioxane in the presence of trimethyl amine as a base to produce the spiro derivative **17** (Scheme 4).



Scheme 2. Preparation of compounds **9a-c** and **11a-c**

Pharmacological activities

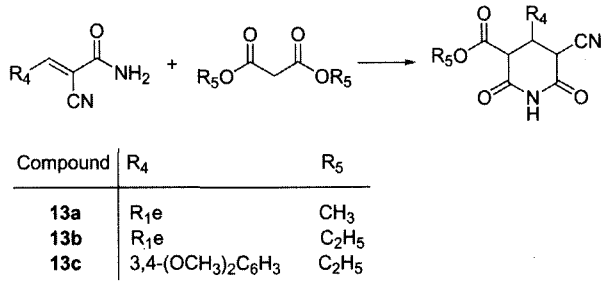
The muscle relaxant, anticonvulsant, and potentiation of phenobarbital-induced sleep are represented in Fig. 1, Tables III and IV.

Muscle relaxant activity (traction test)

All the test compounds exhibited potent muscle relaxant activity as compared to normal saline. The data shown in Fig. 1 indicate that the order of muscle relaxation potency in decreasing order is compound **6**, followed by compound **13a**, then compounds **13c** and **13b**.

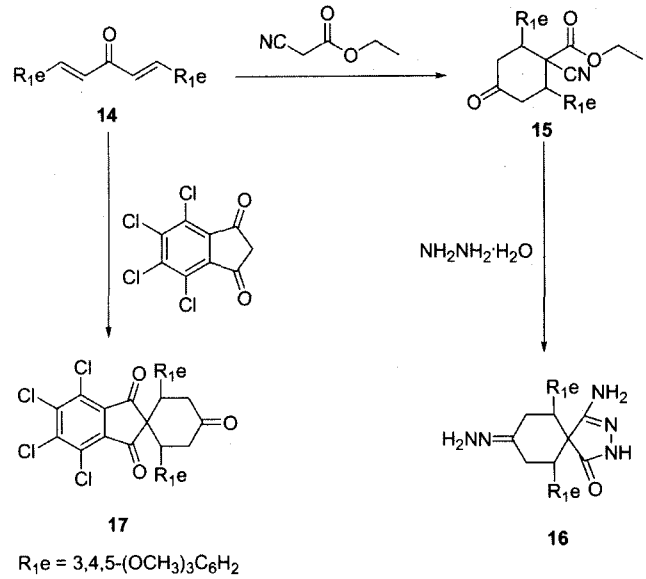
Anticonvulsant activity (strychnine-induced seizure test)

Strychnine is a central stimulant acting by blocking the receptors for the inhibitory transmitter, glycine (Bloom,



Compound	R_4	R_5
13a	R_1e	CH_3
13b	R_1e	C_2H_5
13c	3,4-$(OCH_3)_2C_6H_3$	C_2H_5

Scheme 3. Preparation of compounds **13a-c**



Scheme 4. Preparation of compounds **16** and **17**

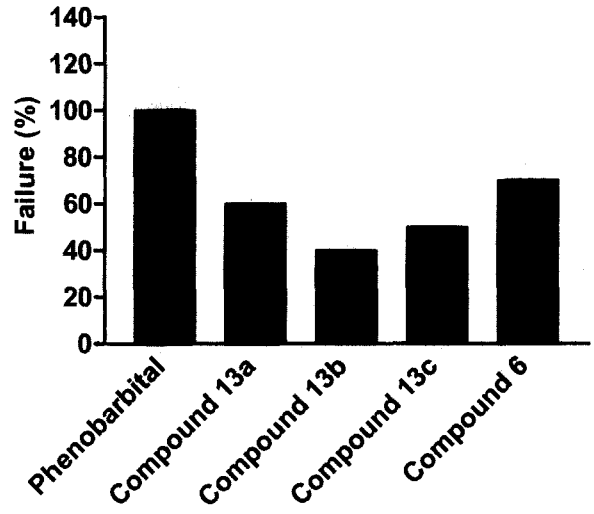


Fig. 1. Effect of test compounds on muscle relaxant activity by traction test

Table IV. Potentiation of Phenobarbital sodium-induced sleep by the test compounds in mice

Treatment	Dose (mg/kg)	Latency (min)	Duration (min)
Phenobarbital	90	46.8 ± 0.73	134.5 ± 1.34
Compound **13a**	50	27.3 ± 0.56*	186.4 ± 1.82*
Compound **13b**	50	28.8 ± 0.62*	193.1 ± 0.71*
Compound **13c**	50	21.7 ± 1.72*	204.1 ± 1.69*
Compound **6**	50	24.3 ± 1.16*	194.1 ± 2.58*

Test compounds **13a-c, 6** were injected i.p. one hour prior to the administration of phenobarbital sodium (i.p.). Mean latency and duration of sleep in minutes ± SEM (n=11) are presented.
*Statistically significant difference from the phenobarbital group at *p<0.0001* using Students *t*-test for comparison.

2001). All of the tested compounds showed significant protection against strychnine (0.2 mg/kg) induced seizures at a dose level of 50 mg/kg. The compounds **13a** and **13c** were the most potent causing a 4 fold prolongation in the onset seizure time compared to strychnine. The compounds **13b** and **6** produced a 2 fold prolongation. Regarding the protection against mortality caused by strychnine, the compound **13c** offered maximum protection (83%) while the compound **13b** was the least protective (30%).

Sedative/hypnotic activity (potentiation of phenobarbital sodium-induced sleep)

The screening of the tested compounds for sedative and hypnotic activities revealed that they all possess potent sedative and hypnotic activities.

All the compounds (50 mg/kg) significantly ($p<0.0001$) decreased the onset of phenobarbital (90 mg/kg)-induced sleep as well as prolonged the sleep duration. Concerning the reduction in sleep onset, compound **13c** was the most potent, followed by compound **6**, then compounds **13a** and **13b** (equipotent). From the results, it appears that the substitution of the carbonyl group at position **6** with a metamethyl phenyl group (compounds **13b** versus **6**) significantly reduced the onset of sleep ($p<0.05$). The addition of a third methoxy group on the (phenyl group) at position 4 seems to be related to the reduction in hypnotic activity (compound **6** versus compound **13c**).

REFERENCES

Aboal-Enein, M. N. and El-Azzouny, A. A., A Facile Synthesis of 2',3',7',8'-Tetrahydro-7'-Aryl and Aralkylspiro[cycloalkyl 1,6' (5'*H*)-imidazo[1,2-a] pyrazin]-5'-ones. A Heterocycle of Potential Analgesic Profile. *Egypt. J. Chem.*, 31, 141 (1988).

Allen, L. E., Ferguson. H. C., and Kissel, J. W., Psychosedative Agents 2,8-(4-substituted-1-piperazinylalkyl)-8-azaspiro-[4.5]-decane-7,9-diones. *J. Med. Chem.*, 15, 477 (1972).

Bloom, F., Neurotransmission and the Central nervous system. In *the Pharmacological basis of therapeutics.* Hardman J, Limbird L, Goodman A (eds). 10th Edition McGraw Hill Companies Jne. New York, USA (2001).

Bose, M., Dutta, D., Pathak, B., and Patra, B., Synthesis of

Table III. Anticonvulsant activities of the test compounds against strychnine-induced seizure in mice

Treatment	Dose (mg/kg)	Onset of seizure (min)		Seizure protection (%)		Mortality (%)	
		0.5 h	1 h	0.5 h	1 h	0.5 h	1 h
Strychnine	0.2	2.8 ± 0.11	2.8 ± 0.11	0	0	100	100
Phenobarbital	200	5.5 ± 0.22*	2.4 ± 0.05*	50	80	0	0
Compound **13a**	50	3.75 ± 0.63	11.0 ± 2.00*	20	66	80	34
Compound **13b**	50	4.5 ± 0.65*	7.0 ± 0.82*	20	30	80	70
Compound **13c**	50	6.8 ± 2.23	10.3 ± 0.35*	20	83	80	17
Compound **6**	50	4.3 ± 1.32	5.7 ± 0.33*	20	50	80	50

Test compounds **13a-c, 6** were injected i.p. 30, and 60 minutes prior to the administration of strychnine (0.2 mg/kg i.p.). Values are the mean ± SEM (n=10).
* Statistically significant difference compared to the strychnine treated group (*P<0.05*) using Students *t*-test.

some spiro[5.5] undecanes as possible Analgesic agents. *J. Indian Chem. Soc.*, 62, 69 (1985).

Braulio, I, Alfredo, P., John, V., and Jairo, Q., Synthesis of 2,3-dihydropyrido and 2,3-dihydropyrimido[1,4] diazepines from triamino pyridine and triamino pyrimidine. *J. Heterocyclic Chem.*, 34, 1555 (1997).

Carrol, F. A., Prespectives on structure and Mechanism. In *Organic Chemistry.* Brooks/Cole Publishing Company 3rd Edition 645 (1988).

Collins, I., Moyes, C., Davey, W., Rowley, M., Bromidge, F., Quirk, K., Atack, J., McKernan, R., Thompson, S. A., Wafford, K., Dawson, G., Pike, A., Sohal, B., Tsou, N., Bull, R., and Castro, J., 3-Heteroaryl-2-pyridones: Benzodiazepine site ligands with functional selectivity for α2/α3-subtypes of human $GABA_A$ receptor-ion channels. *J. Med. Chem.*, 45, 1887-1900 (2002).

De, A. U. and Pal, D., Possible Antineoplastic Agents 11., *J. Pharm. Sci.*, 66, 232 (1977).

Goehring, R. R, Greenwood, T. D., Nwokogus, G. C., Pisipati, J. S., Rogers, T. G., Wolfe, J. F. (Harvey W. Peters Res Cent. Study Parkinsons Dis. Disord. Cent. Nerv. Syst. Virginia Polytechnic Inst. And state Univ. Blacksburg. VA 24061 USA), Synthesis and anticonvulsant activity of 2-benzylglutarimides. *J. Med. Chem.*, 33 (3), 926 (Eng) (1990). T.C.A. 112, 118615p (1990).

James, A. R., Gordon, D. J., Witold, F. K., Teanby, H. S., and John, W. P. (wellcome Foundation Ltd.), PCT Int. Appl. WO 95 01326 (Cl. Co7 C233/25), 12 Jan 1995, GB Appl. 93/13, 459, 30 Jun 1993; 60pp., Preparation of antiatherosclerotic diaryl compounds; T.C.A. 123, 143447V. (1995).

March, J., Advanced Organic Chemistry. John Willey and Sons, 4th Edition, 741 (2000).

Meyers, F. H, Jawetz, E., and GoldFien, A., Review of Medicinal Pharmaacology., Lange Medical Publications, Los Atlos, California; 5th Edition, 222 (1976).

Osman, A. N., Kandeel, M. M., Said, M. M., and Ahmed, E. M., Synthesis and anticonvulsant activity of some spiro compound derived from barbituric and thiobarbituric acids. *Indian J. Chem.*, 35 B, 1073 (1996).

Reddy, D. B., Padmavathi, V., and Reddy, P. V. R., Cyclokexanone dicarboxylates as precursors for the synthesis of some spiropyrimidinetetrones and spiropyrazolidinetriones. *Indian J. Chem.*, 31 B, 774 (1992).

Reddy, T. I. and Varma, R. S., Rare-earth (RE) exchanged nay zeolite promoted Knoevenagel condensation. *Tetrahedron Lett.*, 38, 1721 (1997).

Sergio, M., Tiziano, B., Enzo, B., Metlide, B. (Farmitalia Carlo Erba S.R.I.) Eur. Pat. Appl. Ep 449, 346 (Cl CO7D 457/02), 02 Oct 1991. GB Appl. 9016, 722, 27 Mar 1990; 9 pp.; preparation of [(4.piperidinyl)akyl] ergolines as antiparkinson agents; T.C.A. 116, 21303e (1992).

Sykes, P., A guidebook to Mechanism in Organic Chemistry Long mon London and New York, New York, 5th Edition, 198 (1985).

Villar, R., Laguna, M. R., Calleja, J. M., and Cadavid, I., Effect of Kseletonema statum extracts on the central nervous system. *Planta Med.*, 38, 398 (1992).

Vogel, A. G. and Vogel, W. H., Drug Discovery and Evaluation, Pharmacological Assay, Spring, Berlin 260 (1997).

Vollhardt, K. P. C., Organic Chemistry., W. H. Freeman and Company New York 3rd Edition, 1048 (1999).