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SYNTHESIS AND EVALUATION OF THE PHARMACOLOGICAL ACTIVITY OF OPEN ANALOGUES OF CROMAKALIM CARRYING UREA AND THIOUREA MOIETIES

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ABSTRACT

Some new ring-opened analogues of cromakalim bearing urea and thiourea moieties were synthesized and tested as vasodilators rat trachea and aorta of respectively, and as stimulators of elastin synthesis from isolated humain vascular smooth muscle cells. Cromakalim, pinacidil, diazoxide, and verapamil were used as refrences compounds in the vasodilating experiments while diazoxide was used as a reference in the elastin experiments. Furthur investigations has been undertaken to determine the mechanism of action of the vasodilator activity.The pharmacological results on rat aorta rings revealed that the most active vasodilator compound was **4a**, which showed ED_{50} value of 1.5 M, and was almost 15-fold more active than diazoxide, but was 4-fold and 11 folds less active than pinacidil and cromakalim respectively. Further investigations revealed that **2n** was a clear ATP-sensitive potassium channel activator like diazoxide and cromakalim, while **2m**, **2j**, **3f**, and **4e** werevoltage-gated calcium channel blockers like verapamil. Finally, **3g** and **3i** could be considered as partial ATP-sensitive potassium channel activators. Further investigation on rat trachea rings revealed that **4e** was interestingly 21-fold more active than cromakalim but was non-tissue-selective. Investigations on elastin synthesis showed that diazoxide significantly elevated elastin quantity by 34% at 50 µM. Compound **2d** (20µM) increased elastin production by 21%, which represents approximately 61% of the effect of 50µM diazoxide, and **3g** (20µM) increased elastin production by 28%, which is around 82% of the effect induced by 50 µM diazoxide, while at the highest concentrations (50-100 µM) **3g** reduced elastin production.

Keywords: Ring-opened cromakalimanalogues, Voltage-gated calcium channel blockers.

INTRODUCTION

In the last few decades, pharmacology and chemistry of benzopyrans, chromone and coumarin-apparented molecules, had generateda great interest of researchers worldwide, in order to develop new bioactive compounds[1-5].This interest is due to the prevalence of benzopyran system in many natural and synthetic bioactive compounds. Indeed, several new structures have been developed, covering different pharmacological aspects. For example, new hybrid compounds between vitamin E and class I and class III antiarrhythmic drugs were reported, giving

benzopyran analogs, as novel antiarrhythmics against ischemia-reperfusion injury (Figure 1)[6-9].

Some of these analogs showed preventing properties against reperfusion arrhythmias, which could be attributed to their combined inhibition of free radical–mediated damage and antiarrhythmic properties. Potassium channel openers (PCOs) comporting benzopyran system have been reported to activate ATP-sensitive potassium (K_{ATP}) channels. Thus, according to their tissue selectivity, PCOs may be expected to become new therapeutic agents for diseases such as type 1 or 2 diabetes, obesity, hyperinsulinism, arterial hypertension, angina pectoris, bronchial asthma, and urinary incontinence (Figure 1)[10-12].The most known synthetic leader of benzopyran as PCO is cromakalim, which has been largely studied in term of pharmacology and structural modulation to obtain new therapeutic agents, acting on cardiovascular system (Figure 2)[10,13-19]. Recently, new works have revealed that some newcromakalim analogues exerted vasodilator activity on vascular smooth muscles (rat aorta and trachea), not by activation of K_{ATP}, but by blocking voltage-gated calcium channels (VGCC) (Figure 2)[20].This discovery has been confirmed by our own work on new ring-opened analogues of cromakalimrecently developed in our laboratory (Figure 2)[21].

Furthermore, these new blockers of VGCC, resulting from the ring opening of cromakalim, also showed interesting elastin-stimulating activity on cultured rat vascular smooth muscle, and presented a structural similarity with verapamil, a well known VGCC blocker (Figure 2)[21].The implication of VGCCs in the stimulation of elastin synthesis has been previously confirmed[22].Based on these previous data, we proposed in the present studytheinvestigation ofnewring-opened derivativesof cromakalimbearingurea and thiourea moieties, instead of sulfonylurea moieties, which will present one unic form at physiological pH (Scheme 1, compounds **2a-r**, **3a-n** and **4a-l**). Indeed, we found in our recent workthat the N-methylated sulfonylurea derivatives were much more active than the unmethylated ones, due to the weak acidic character of the later molecules (Figure 2)[21]. The newly synthesized compounds were pharmacologically investigated using three models,the vascular (aortic rings) and respiratory (trachea) smooth muscles of rat precontracted by 30 mMKCl, in order to evaluate theirvasodilator activity and their eventual tissue-selectivity, and the isolated humainvascular smooth muscle cells (VSMCs,), in order to evaluate the eventual stimulation of elastin synthesis by target compounds. The most active compounds were tested again on the aortic ring model, in the presence of glibenclamide, a K_{ATP} channel blocker, or 80mM KCl, in order to determine the mechanism(s) of action of these compounds.

EXPERIMENTAL

Chemistry

Melting points were determined on Buchi-Tottoti capillary apparatus and are uncorrected. IR spectra were recorded as KBr pellets on a Perkin-Elmer 1750 FT spectrophotometer. The ^{1}HNMR spectra were recorded on a Brüker (500 and 400 MHz) in DMSO-d_6 or in $CDCl_3$with hexamethyldisiloxane (HMDS) as an internal standard; chemical shifts are reported in δ values relative to internal HMDS.

General procedure for the synthesis of 2a-r, 3a-n and 4a-n

A mixture of aryl (or alkyl) isocyanate (or isothiocyanate) (1.1 eq) andcommercial amine **2a**(**2b**, **2c**) or previousely described amine **1d-l** (1eq)[21]in dichloromethan (20 ml)was stirred at room temperature for 30 minutes. The white precipitate formed was filtred under vacuum, washed with a small amount of diethyl ether and dried.

1-Butyl-3-(2-methoxybenzyl) urea (2a)

White powder (92%)**.** mp124 °C.IR (KBr 2%, v cm^{-1}): 3355, 3340, 3120, 1525, 1635, 3060, 3020,2865.^{1}H NMR (DMSO-d_6, 500MHz, δ ppm, J Hz) : 0.86 (t, 3H, $CH_2CH_2CH_2\mathbf{CH_3}$, J = 10Hz), 1.26 (m, 2H, $CH_2CH_2\mathbf{CH_2}CH_3$), 1.34 (m, 2H, $CH_2\mathbf{CH_2}CH_2CH_3$), 2.99 (q, 2H, $\mathbf{CH_2}CH_2CH_2CH_3$, J = 10Hz), 3.79 (s, 3H, OCH3), 4.15 (d, 2H, $NH\mathbf{CH_2}$, J = 10Hz), 5.92 (s, 1H, NH, J = 7.5Hz), 6.04 (s, 1H, NH, J = 10Hz), 6.89 (t, 1H, CHarom, J = 10Hz), 6.95 (d, 1H, CHarom, J = 10Hz), 7.15 ((dd, 1H, CHarom, J = 5Hz, 10Hz), 7.21 (td, 1H, CHarom, J = 5Hz, 10Hz). ^{13}C NMR (DMSO-d_6, 125MHz, δ ppm, J Hz) : 14.16 ($CH_2CH_2CH_2\mathbf{C}H_3$), 19.98 ($CH_2CH_2\mathbf{C}H_2CH_3$), 32.62 ($CH_2\mathbf{C}H_2CH_2CH_3$), 38.60 ($NH\mathbf{C}H_2$), 39.38 ($\mathbf{C}H_2CH_2CH_2CH_3$), 55.71 ($O\mathbf{C}H_3$), 110.83 ($C_{arom}$), 120.54 ($C_{arom}$), 128.17 ($C_{arom}$), 128.27 ($C_{arom}$), 128.81 ($C_{arom}$), 157.14 ($O\mathbf{C}_{arom}$), 158.55 ($\mathbf{C}$=O).Anal. Calculated (%) for $C_{13}H_{20}N_2O_2$: C, 66.07; H, 8.53; N, 11.85; found: C, 66.08; H, 8.55; N**,** 11.84.

1-Butyl-3-(*2*-ethoxybenzyl) urea (2b)

White powder (92%). mp 112-114 °C.IR (KBr 2%, v cm^{-1}): 3360, 3320, 1530;1630;3040; 2930. ^{1}H NMR (DMSO-d_6, 500MHz, δ ppm, J Hz): 0.87 (1t, 3H, $CH_2CH_2CH_2\mathbf{CH_3}$, J =10Hz),

1,265 (sixtuplet, 2H, $CH_2CH_2\underline{\mathbf{CH_2}}CH_3$, J = 7Hz), 1,345 (m, 5H, $CH_2\underline{\mathbf{CH_2}}CH_2CH_3$ + $OCH_2\underline{\mathbf{CH_3}}$), 2.99 (1q, 2H, $NH\underline{\mathbf{CH_2}}CH_2CH_2CH_3$, J = 5Hz, 10Hz), 4.04 (1q, 2H, CH_2, J = 10Hz), 4.155 (1d, 2H, $NH\underline{\mathbf{CH_2}}$, J= 5Hz), 5.94 (1t, 1H, NH= 5Hz), 6.00 (1t, 1H, NH, = 5Hz), 6.87 (1t, 1H, CHarom, J= 10Hz), 6.93 (1d, 1H, CHarom, J= 10Hz), 7.155 (t, 1H, CHarom, J= 10Hz), 7.19 (1t, 1H, CHarom, J= 10Hz).Anal. Calculated (%) for $C_{14}H_{22}N_2O_2$: C, 67.17; H, 8.86; N, 11.19; found: C, 67.21; H, 8.84; N, 11.20.

1-(2-Methoxybenzyl)-3-isopropylurea (2c)

White powder (94%). mp 176-180 °C.IR (KBr 2%, v cm^{-1}): 3360, 3300, 3120, 1530;1630;3020;2835. ^{1}H NMR(DMSO-d_6, 500MHz, δ ppm, J Hz) :1.02 (d, 6H, $CH\underline{\mathbf{(CH_3)_2}}$, J = 10Hz), 3.65 (septuplet, 1H, $\underline{\mathbf{CH}}(CH_3)_2$, J = 10Hz), 3.79 (s, 3H, OCH_3), 4.15 (d, 2H, $\underline{\mathbf{CH_2}}NH$, J = 5Hz), 5.79 (d, 1H, NH, J = 5 Hz), 5.95 (d, 1H, NH, J = 7.5 Hz), 6.89 (t, 1H, CHarom, J = 10Hz), 6.95 (d, 1H, CHarom, J = 10Hz), 7.15 (d, 1H, CHarom, J = 10Hz), 7.21 (td, 1H, CHarom, J = 5Hz, 10Hz).^{13}C NMR (DMSO-d_6, 125MHz, δ ppm) : 23.74 ($CH\underline{\mathbf{(CH_3)_2}}$), 38.52 (CH2), 41.37 (CH), 55.72 (OCH_3), (110.85 (C_{arom}), 120.55 (C_{arom}), 128.23 (C_{arom}), 128.29 (C_{arom}), 128.80 (C_{arom}), 157.16 ($O\underline{\mathbf{C}}_{arom}$), 157.86 ($\underline{\mathbf{C}}$=O).Anal. Calculated (%) for $C_{12}H_{18}N_2O_2$: C, 64.84; H, 8.16; N, 12.60; found: C, 64.82; H, 8.14; N**,** 12.80.

1-(2-Ethoxybenzyl)-3-isopropylurea (2d)

White powder (94%). mp 154 °C.IR (KBr 2%, v cm^{-1}): 3330, 3180, 3120, 1530,1635,3040, 2960. ^{1}H NMR (DMSO-d_6, 500MHz, δ ppm, J Hz) : 1.025 (d, 6H, $CH\underline{\mathbf{(CH_3)_2}}$), J = 5Hz), 1.35 (t, 3H, $OCH_2\underline{\mathbf{CH_3}}$, J = 10Hz), 3.67 (septuplet, 1H, , $\underline{\mathbf{CH}}(CH_3)_2$, J = 5Hz), 4.035 (q, 2H, $O\underline{\mathbf{CH_2}}CH_3$, J = 10Hz), 4.155 (d, 2H, $\underline{\mathbf{CH_2}}NH$, J = 5Hz), 5.805 (d, 1H, NH, J = 5Hz), 5.92 (t, 1H, NH, J = 5Hz), 6.875 (td, 1H, CHarom, J = 5Hz, 7.5Hz), 6.93 (d, 1H, CHarom, J = 10Hz), 7.175 (m, 2H, CHarom). ^{13}C NMR (DMSO-d_6, 125MHz, δ ppm) :15.18 ($OCH_2\underline{\mathbf{CH_3}}$), 23.73 ($CH(\underline{\mathbf{C}}H_3)_2$), 38.35 ($\underline{\mathbf{C}}H_2NH$), 41.38 $\underline{\mathbf{C}}H(CH_3)_2$), 63.63 ($O\underline{\mathbf{C}}H_2CH_3$), 111.78 ($C_{arom}$), 120.46 ($C_{arom}$), 128.20 ($C_{arom}$), 128.21 ($C_{arom}$), 129 ($C_{arom}$), 156.44 ($O\underline{\mathbf{C}}_{arom}$), 157.88 ($\underline{\mathbf{C}}$=O). Anal. Calculated (%) for $C_{13}H_{20}N_2O_2$: C, 66.07; H, 8.53; N, 11.85; found: C, 66.05; H, 8.52; N, 11.86.

1-(2-Metoxybenzyl)-3-tert-butylurea (2e)

White powder (92%). mp 146 C°.IR (KBr 2%, v cm^{-1}):3360, 3320, 1525; 1638; 3020,2825.^{1}H NMR (DMSO-d_6, 500MHz, δ ppm, J Hz): 1.22 (s, 9H, $C\underline{\mathbf{(CH_3)_3}}$), 3.79 (s, 3H, OCH_3), 4.12 (d, 2H, CH_2, J = 10Hz), 5.77 (s, 1H, NH), 5.91 (t, 1H, NH, J = 10Hz), 6.89 (t, 1H, CHarom, J = 10Hz), 6.95 (d, 1H, CHarom, J = 10Hz), 7.155 (d, 1H, CHarom, J = 10Hz), 7.21 (t, 1H, CHarom, J = 10Hz). ^{13}C NMR (DMSO-d_6, 125MHz, δ ppm) :29.80 ($C(\underline{\mathbf{C}}H_3)_3$), 38.27 ($CH_2$), 49.48 80 ($\mathbf{C}(CH_3)_3$), 57.71 ($OCH_3$), 110.85 ($C_{arom}$), 120.57 ($C_{arom}$), 128.29 ($C_{arom}$), 128.86 ($C_{arom}$), 157.22 ($O\underline{\mathbf{C}}_{arom}$), 157.80 ($\underline{\mathbf{C}}$=O).Anal. Calculated (%) for $C_{13}H_{20}N_2O_2$: C, 66.07; H, 8.53; N, 11.85; found: C, 66.05; H, 8.52; N, 11.83.

1-(2-Etoxybenzyl)-3-tert-butylurea (2f)

White powder (92%). mp 145 °C.IR (KBr 2%, v cm^{-1}):3380, 3300, 3120, 1510,1630,3040, 2975. ^{1}H NMR (DMSO-d_6, 500MHz, δ ppm, J Hz):1.22 (s, 9H, $C(CH_3)_3$), 1.35 (t, 3H, $OCH_2\underline{\mathbf{CH_3}}$, J = 10Hz), 4.035 (q, 2H, $O\underline{\mathbf{CH_2}}CH_3$, J =10Hz), 4.125 (d, 2H, $NH\underline{\mathbf{CH_2}}$, J = 5Hz), 5.78 (s, 1H, NH), 5.87 (t, 1H, NH, J = 5Hz), 6.88 (t, 1H, CHarom, J = 10Hz), 6.93 (d, 1H, CHarom, J = 10Hz), 7.17 (m, 2H, CHarom).^{13}C NMR (DMSO-d_6, 125MHz, δ ppm,) :15.18 ($OCH_2\underline{\mathbf{C}}H_3$), 29.81 ($C(\underline{\mathbf{C}}H_3)_3$), 38.25 ($NH\underline{\mathbf{C}}H_2$), 49.49 ($\underline{\mathbf{C}}(CH_3)_3$), 63.63 ($O\underline{\mathbf{C}}H_2CH_3$), 111.78 ($C_{arom}$), 120.48 ($C_{arom}$), 128.21 ($C_{arom}$), 129.07 ($C_{arom}$), 156.47 ($O\underline{\mathbf{C}}_{arom}$), 157.82 ($\underline{\mathbf{C}}$=O).Anal. Calculated (%) for $C_{14}H_{22}N_2O_2$: C, 67.17; H, 8.86; N, 11.19; found: C, 67.20; H, 8.85; N**,** 11.20.

1-(2-Methoxybenzyl)-3-allylurea (2g)

White powder (93%). mp 138 °C.IR (KBr 2%, v cm^{-1}):3360, 3120, 1530;1630;3000, 3015; 2840.^{1}H NMR (DMSO-d_6, 500MHz, δ ppm, J Hz):3.64 (1t, 2H, CH_2, J = 5.43),3.64 (t, 2H, $NH\underline{\mathbf{CH_2}}CH{=}CH_2$, J = 5Hz), 3.79 (1s, 3H, OCH_3), 4.165 (1d, 2H, $\underline{\mathbf{CH_2}}NH$, J= 5Hz), 5.015 (1dd, 1H, CH_{vinyl}, 5Hz, J= 10Hz), 5.11 (1dd, 1H, CH_{vinyl}, J= 5Hz, 17.5Hz), 5,81 (1m, 1H, CH), 6.06 (1t, 1H, NH, J= 5Hz), 6.15 (1t, 1H, NH, J= 5Hz), 6.89 (1t, 1H, CHarom, J= 10Hz), 6.96 (1d, 1H, CHarom, J= 10Hz), 7.165 (1d, 1H, CHarom, J= 5Hz), 7.215 (1t, 1H, CHarom, J= 10Hz). Anal. Calculated (%) for $C_{12}H_{16}N_2O_2$: C, 65.43; H, 7.32; N, 12.72; found: C, 65.45; H, 7.30; N**,** 12.70.

1-(2-Ethoxybenzyl)-3-allylurea (2h)

White powder (92%). mp 135 °C.IR (KBr 2%, v cm^{-1}): 3320, 3130, 1540;1630;3040; 2980.^{1}H NMR (DMSO-d_6, 500MHz, δ ppm, J Hz) : 1.35 (t, 3H, $OCH_2\underline{\mathbf{CH_3}}$, J = 10Hz), 3.654 (m, 2H, $\underline{\mathbf{CH_2}}CH{=}CH_2$), 2.02 (q, 2H, $O\underline{\mathbf{CH_2}}CH_3$, J = 10Hz), 4.175 (d, 2H, $NH\underline{\mathbf{CH_2}}$, J = 5Hz), 5.02 (m, 1H, CHvinyl), 5.11 (m, 1H, CHvinyl), 5.815 (m, 1H, $CH_2\underline{\mathbf{CH}}{=}CH_2$), 6.105 (q, 2H, 2NH, J = 5Hz), 6.88 (t, 1H, CHarom, J = 10Hz), 6.94 (d, 1H, CHarom, J = 10Hz), 7.18 (m, 2H, CHarom).^{13}C NMR (DMSO-d_6, 125MHz, δ ppm) :15.17 ($OCH_2\underline{\mathbf{CH_3}}$), 38.70 ($NH\underline{\mathbf{C}}H_2$), 42.18 ($\underline{\mathbf{C}}H_2CH{=}CH_2$), 63.65 ($O\underline{\mathbf{C}}H_2CH_3$),

111.79 (C_{arom}), 114.76 ($CH_2CH=\underline{C}H_2$), 120.46 (C_{arom}), 128.18 (C_{arom}), 128.26 (C_{arom}), 128.86 (C_{arom}), 137.31 ($CH_2\underline{C}H=CH_2$), 156.42 ($O\underline{C}_{arom}$), 158.35 ($\underline{C}$=O).Anal. Calculated (%) for $C_{13}H_{18}N_2O_2$: C, 66.64; H, 7.74; N, 11.96; found: C, 66.65H, 7.75; N**,** 11.95.

1-(2-Methoxybenzyl)-3-heptylurea (2i)

White powder (92%); mp 118 °C. IR (KBr 2%, v cm^{-1}) : 3320, 3120, 1520; 1625;3020; 2860.^{1}H NMR (DMSO-d_6, 500MHz, δ ppm, J Hz): 0,86 (t, 3H, CH_3, J = 10 Hz), 1.30 (m, 10H, $(CH_2)_5$), 2.98 (q, 2H, CH_2, J = 10Hz), 3.79 (s, 3H, OCH_3), 4.14 (d, 2H, CH_2, J = 5Hz), 5.91 (t, 1H, NH, J = 7.5Hz), 6.03 (t, 1H, NH, J = 7.5Hz), 6,88 (td, 1H, CHarom, J = 5Hz, 10Hz), 6.95 (d, 1H, CHarom, J = 10Hz), 7.15 (d, 1H, CHarom, J = 10Hz), 7.21 (t, 1H, CHarom, J = 10Hz).).^{13}C NMR (DMSO-d_6, 125MHz, δ ppm) :14.40 ($CH_2\underline{C}H_3$), 22.50 ($\underline{C}H_2CH_3$), 26.81 (CH_2), 28.93 (CH_2), 30.50 (CH_2), 31.75 (CH_2), 38.58 (CH_2), 39.40 (CH_2), 55.72 (OCH_3), 110.82 (C_{arom}), 120.51 (C_{arom}), 128.14 (C_{arom}), 128.25 (C_{arom}), 128.84 (C_{arom}), 157.13 ($O\underline{C}_{arom}$), 158.52 ($\underline{C}$=O). Anal. Calculated (%) for $C_{16}H_{26}N_2O_2$: C, 69.03; H, 9.41; N, 10.06; found: C, 69.00; H, 9.39; N, 10.03.

1-(2-Methoxybenzyl)-3-octylurea (2j)

White powder (94%). mp 112-113 °C.IR (KBr 2%, v cm^{-1}): 3320, 1525; 1625; 3020; 2860.^{1}H NMR (DMSO-d_6, 500MHz, δ ppm, J Hz):0.86 (t, 3H, CH_3, J = 7.5Hz), 1.31 (m, 12H, $(CH_2)_6$), 2.98 (q, 2H, CH_2, J = 7.5Hz), 3.79 (s, 3H, OCH_3), 4.15 (d, 2H, CH_2, j = 10Hz), 5.92 (t, 1H, NH, J = 7.5Hz), 6.04 (t, 1H, NH, J = 7.5Hz), 6.88 (t, 1H, CHarom, J = 10Hz), 6.95 (d, 1H, CHarom, J = 10Hz), 7.30 (d, 1H, CHarom, J = 10Hz), 7.21 (t, 1H, CHarom, J = 10Hz). ^{13}C NMR (DMSO-d_6, 125MHz, δ ppm) : 14.39 (CH_3), 22.56 (CH_2), 26.87 (CH_2), 29.18 (CH_2), 29.25 (CH_2), 30.50 (CH_2), 31.71 (CH_2), 38.59 (CH_2), 39.39 (CH_2), 55.69 (OCH_3), 110.81 (C_{arom}), 120.50 (C_{arom}), 128.13 (C_{arom}), 128.24 (C_{arom}), 128.84 (C_{arom}), 157.13 ($O\underline{C}_{arom}$), 158.54 ($\underline{C}$=O). Anal. Calculated (%) for $C_{17}H_{28}N_2O_2$: C, 69.83; H, 9.65; N, 9.60; found: C, 69.80; H, 9.63; N, 9.60.

1-Benzyl-3-(2-methoxybenzyl) urea (2k)

White powder (94%). mp160 °C.IR (KBr 2%, v cm^{-1}):3335; 3160; 1525; 1635; 3020, 3000;2850. ^{1}H NMR (4 DMSO-d_6,500MHz, δ ppm, J Hz) : 3.79 (s, 3H, OCH_3), 4.195 (d, 2H, CH_2, J = 5Hz), 4.22 (d, 2H, CH_2, J = 10Hz), 6.22 (t, 1H, NH, J = 7.5 Hz), 5.45 (t, 1H, NH, J = 7.5 Hz), 6.89 (t, 1H, CHarom, J = 7.5Hz), 6.96 (d, 1H, CHarom, J = 10Hz), 7.175 (d, 1H, CHarom, J = 5Hz), 7.23 (m, 4H, CHarom), 7.31 (t, 2H, CHarom, J = 7.5 Hz). ^{13}C NMR (DMSO-d_6, 125MHz, δ ppm) : 38.75 ($\underline{C}H_2$), 43.46 ($\underline{C}H_2$), 55.73 ($O\underline{C}H_3$), 110.87 (C_{arom}), 120.55 (C_{arom}), 127.02 (C_{arom}), 127.49 (C_{arom}), 128.19 (C_{arom}), 128.34 (C_{arom}), 128.67 (C_{arom}), 141.35 (C_{arom}), 157.16, ($O\underline{C}_{arom}$), 158.54 ($\underline{C}$=O). Anal. Calculated (%) for $C_{16}H_{18}N_2O_2$: C, 71.09; H, 6.71; N, 10.36; found: C, 71.10; H, 6.70; N, 10.35.

1-Benzyl-3-(2-ethoxybenzyl) urea (2l)

White powder (93%). mp 170-172 °C. IR (KBr 2%, v cm^{-1}):3320; 3100; 1535; 1630; 3040; 2975.^{1}H NMR (DMSO-d_6, 500MHz, DMSO-d_6, δ ppm, J Hz) : 1.35 (t, 3H, $OCH_2\underline{CH_3}$, J = 7.5), 4.035 (q, 2H, $O\underline{CH_2}CH_3$, J = 10Hz), 4.21 (t (two superimposed doublet, 4H, 2 CH_2NH), 6.18 (t, 1H, NH, J = 7.5Hz), 6.47 (t, 1H, NH, J = 7.5Hz), 6.88 (td, 1H, CHarom, J = 5Hz, 10Hz), 6.93 (d, 1H, CHarom, J = 10Hz), 7.215 (m, 5H, CHarom), 7.31 (t, 2H, CHarom, J = 5Hz, 10Hz). ^{13}C NMR (DMSO-d_6, 125MHz, δ ppm) :15.17 ($OCH_2\underline{C}H_3$), 38.79 ($\underline{C}H_2NH$), 43.47 ($\underline{C}H_2NH$), 63.65 ($O\underline{C}H_2CH_3$), 111.79 (C_{arom}), 120.46 (C_{arom}), 127.02 (C_{arom}), 127.49 (C_{arom}), 128.18 (C_{arom}), 128.26 (C_{arom}), 128.67 (C_{arom}), 128.86 (C_{arom}), 141.35 (C_{arom}), 156.44 ($O\underline{C}_{arom}$), 158.56 ($\underline{C}$=O). Anal. Calculated (%) for $C_{16}H_{18}N_2O_2$: C, 71.81; H, 7.09; N, 9.85; found: C, 71.80; H, 7.08; N, 9.84.

1-(2-Methoxybenzyl)-3-(2-methoxy phenyl)urea (2m)

White powder (94%). mp 214 °C.IR (KBr 2%, v cm^{-1}): 3360, 3300, 1520,1638,3020,2825.^{1}H NMR (DMSO-d_6, 500MHz, δ ppm, J Hz):3.82 (s, 6H, $2O\underline{CH_3}$), 4.245 (d, 2H, $\underline{CH_2}NH$, 6.845 (m, 2H, CHarom), 6.91 (t, 1H, CHarom, J = 10Hz), 6.95 (d, 1H, CHarom, J = 10Hz), 6.99 (d, 1H, CHarom, J = 10Hz), 7.14 (t, 1H, NH, J = 5Hz), 7.24 (m, 2H, CHarom), 8.05 (bs, 1H, NH), 8.08 (dd, 1H, CHarom, J = 1Hz, 10Hz). ^{13}C NMR (DMSO-d_6, 125MHz, δ ppm) :55.80 (OCH_3), 110.98 (C_{arom}), 111.05 (C_{arom}), 118.50 (C_{arom}), 120.62 (C_{arom}), 120.92 (C_{arom}), 121.42 (C_{arom}), 128.07 (C_{arom}), 128.56 (C_{arom}), 128.61 (C_{arom}), 130 (C_{arom}), 147.82 ($O\underline{C}_{arom}$), 155.64 ($O\underline{C}_{arom}$), 157.28 ($\underline{C}$=O).Anal. Calculated (%) for $C_{16}H_{18}N_2O_3$: C, 67.12; H, 6.34; N, 9.78; found: C, 67.10; H, 6.35; N, 9.79.

1-(2-Ethoxybenzyl)-3-(2-methoxy phenyl)urea (2n)

White powder (92%)**.** mp 188 °C.IR (KBr 2%, v cm^{-1}): 3370, 3300; 1540, 1655,3030,2955. ^{1}H NMR(DMSO-d_6, 500MHz, DMSO-d_6, δ ppm, J Hz) : 1.36 (t, 3H, $OCH_2\underline{CH_3}$, J = 10Hz), 3.82 (s, 3H, OCH_3), 4.06 (q, 2H, $O\underline{CH_2}CH_3$, J = 10Hz), 4.255 (d, 2H, $NH\underline{CH_2}$, J = 5Hz), 6.835 (dd, 2H, CHarom, J = 5Hz, 11.5Hz), 6.90 (t, 1H, CHarom, J = 10Hz), 6.955 (dd, 2H, CHarom, J = 5Hz, 7.5Hz), 7.10 (t, 1H, NH, J = 5Hz), 7.215

(td, CHarom, J = 5Hz, 12.5Hz), 7.10 (s, 1H, NH), 8.09 (dd, 1H, CHarom, J = 5Hz, 10Hz). ^{13}C NMR (DMSO-d_6, 125MHz, δ ppm) :15.20 ($OCH_2\underline{C}H_3$), 38.43 ($NH\underline{C}H_2$), 56.12 ($O\underline{C}H_3$), 63.72 (($O\underline{C}H_2CH_3$), 111.06 (C_{arom}), 111.92 (C_{arom}), 118.52 (C_{arom}), 120.54 (C_{arom}), 120.92 (C_{arom}), 121.42 (C_{arom}), 128.27 (C_{arom}), 128.48 (C_{arom}), 128.51 (C_{arom}), 130 147.83 (C_{arom}), 155.66 ($O\underline{C}_{arom}$), 156.53 ($\underline{C}$=O).Anal. Calculated (%) for $C_{17}H_{20}N_2O_3$: C, 67.98; H, 6.71; N, 9.33; found: C, 67.99; H, 6.70; N, 9.32.

1-(*2*-Methoxybenzyl)-3-(*3,5*-dimethyl phenyl)urea (2o)

White powder (92%). mp 250 °C.IR (KBr 2%, v cm^{-1}): 3360, 3280, 3200, 3170, 3100, 1540, 1660,3030, 2835. ^{1}H NMR(DMSO-d_6, 500MHz, δppm, J Hz):2.19 (1s, 6H, $O\underline{CH_3}$); 3.82 (1s, 3H, $O\underline{CH_3}$); 4.235 (1d, 2H, $NH\underline{CH_2}$, J= 5Hz); 6.37 (1t, 1H, NH, J= 5Hz); 6.53 (1s, 1H, CHarom); 6.91 (1t, 1H, CHarom, J= 7.5Hz); 6.99 (1d, 1H, CHarom, = 10Hz); 7.00 (s, 2H, CHarom), 7.235 (m, 2H, CHarom), 8.37(1s, 1H, NH).Anal. Calculated (%) for $C_{17}H_{20}N_2O_2$: C, 71.81; H, 7.09; N, 9.85; found: C, 71.80; H, 7.08; N, 9.84.

1-(*2*-Methoxybenzyl)-3-(*3*-methoxy phenyl)urea (2p)

White powder (92%). mp144 °C.IR (KBr 2%, v cm^{-1}): 3350, 3320, 3270, 3200, 3130, 1535, 1660, 3020, 3000, 2835.^{1}H NMR (DMSO-d_6, 500MHz, δ ppm, J Hz) : 3.79 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 4.425 (d, 2H, $NH\underline{CH_2}$, J = 5Hz), 6.40 (bs, 1H, NH), 6.47 (d, 1H, CHarom, J = 10Hz), 6.85 (d, 1H, CHarom, J = 10Hz), 6.91 (t, 1H, CHarom, J = 10Hz), 6.99 (d, 1H, CHarom, J = 10Hz), 6.11 (d, 2H, CHarom), 7.235 (m, 2H, CHarom), 8.85 (bs, 1H, NH).^{13}C NMR (DMSO-d_6, 125MHz, δ ppm) :38 .65 ($NH\underline{C}H_2$), 55.30 ($O\underline{C}H_3$), 55.81 ($O\underline{C}H_3$), 103.83 (C_{arom}), 106.99 (C_{arom}), 110.42 (C_{arom}), 110.98 (C_{arom}), 120.63 (C_{arom}), 128.06 (C_{arom}), 128.50 (C_{arom}), 128.62 (C_{arom}), 129.84 (C_{arom}), 142.19 (C_{arom}), 155.53 ($\underline{C}$=O), 157.29 ($O\underline{C}_{arom}$), 160.14 ($O\underline{C}_{arom}$). Anal. Calculated (%) for $C_{16}H_{18}N_2O_3$: C, 67.12; H, 6.34; N, 9.78; found: C, 67.09; H, 6.31; N, 9.80.

1-(*2*-Methoxybenzyl)-3-phenylurea (2q)

Whitepowder (93%).mp 172-174 °C.IR (KBr 2%, v cm^{-1}): 3320, 3280, 3175, 1520, 1638, 3020, 2835.^{1}H NMR (DMSO-d6, 500MHz, , δ ppm, J Hz):3.83 (s, 3H, OCH_3), 4.26 (d, 2H, CH_2, J = 10Hz), 6.41 (t, 1H, NH, J = 7.5Hz), 6.88 (t, 1H, CHarom, J = 10Hz), 6.91 (t, 1H, CHarom, J = 10Hz), 6.99 (d, 1H, CHarom, J = 10Hz), 7.23 (m, 4H, CHarom), 7.39 (d, 2H, CHarom, J = 10Hz).^{13}C NMR (DMSO-d_6, 125MHz, δ ppm) : 38.66 (CH_2), 55.80 (OCH_3), 110.98 (C_{arom}), 118.05 (C_{arom}), 120.64 (C_{arom}), 121.49 (C_{arom}), 128.10 (C_{arom}), 128.51 (C_{arom}), 128.61 (C_{arom}), 129.11 (C_{arom}), 140.97 (C_{arom}), 155.63 ($O\underline{C}_{arom}$), 157.29 ($\underline{C}$=O).Anal. Calculatedd (%) for $C_{15}H_{16}N_2O_2$: C, 70.29; H, 6.29; N, 10.93; found: C, 70.30; H, 6.30; N, 10.91.

1-(*2*-Ethoxybenzyl)-3-phenylurea (2r)

White powder (92%); mp 174-176 °C.IR (KBr 2%, v cm^{-1}):3335, 3190, 3100, 1535, 1635, 3040, 2975.^{1}H NMR (DMSO-d6, 500MHz, , δ ppm, J Hz):1.37 (t, 3H, $OCH_2\underline{CH_3}$), 4.065 (q, 2H, $O\underline{CH_2}CH_3$, J = 12.5Hz), 4.265 (d, 2H, $\underline{CH_2}NH$, J = 5Hz), 6.36 (t, 1H, $CH_2\underline{NH}$, J = 5Hz), 6.89 (m, 2H, CHarom), 6.96 (d, 1H, CHarom, J = 10Hz), 7.215 (m, 4H, CHarom), 7.39 (d, 2H, CHarom, J = 10Hz), 8.56 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 125MHz, δ ppm) :15.19 (CH_3), 38.68 ($NH\underline{C}H_2$), 63.71 ($O\underline{C}H_2CH_3$), 111.90 (C_{arom}), 118.06 (C_{arom}), 120.54 (C_{arom}), 121.48 (C_{arom}), 128.29 (C_{arom}), 128.48 (C_{arom}), 128.53 (C_{arom}), 129.11 (C_{arom}), 140.98 (C_{arom}), 155.64 ($O\underline{C}_{arom}$), 156.55 ($\underline{C}$=O). Anal. Calculated (%) for $C_{16}H_{18}N_2O_2$: C, 71.09; H, 6.71; N, 10.36; found: C, 71.06; H, 6.70; N, 10.37.

1-(2-Methoxybenzyl)-3-cyclohexylthiourea (3a)

White powder (56,74 %). mp: 118-120°C. IR (KBr 2%, v cm^{-1}):3290, 3110, 1556,1580,3020,2860. ^{1}H NMR (DMSO-d_6, 500MHz, δ ppm, J Hz):1.16 (m, 4H, $2CH_2$), 1.27 (m, 2H, CH_2), 1.54 (m, 1H, CH), 1.65 (m, 2H, CH_2), 1.85 (m, 2H, CH_2), 3.81 (1s, 3H, OCH_3),3.98 (brs, 1H, NH), 4.58 (s, 2H, CH_2),6.91 (t, 1H, CH_{arom}, J= 7.5, 1.5),6.99 (d, 1H, CH_{arom}, J= 8),7.17 (d, 1H,CH_{arom}, J= 7),7.25 (t, 1H, CH_{arom}, J= 7.5, 1.5),7.40 (brs, 1H, NH), 9.66 (brs, 1H, NH). Anal. Calculated (%) for $C_{15}H_{21}N_2OS$: C, 64.94; H, 7.63; N, 10.10; found: C, 64.48; H, 8.02; N, 10.42.

1-(2-Methoxybenzyl)-3-isopropylthiourea (3b)

White powder (65,72 %). mp: 112 °C. IR (KBr 2%, v cm^{-1}):3300,1560, 3010, 2900. ^{1}H NMR (DMSO-d_6, 500MHz, δ ppm, J Hz):1.03 (1d, 6H, $2CH_3$, J= 6.5), 3.34 (1m, 1H, CH, J= 6.60, 6.72, 6.92), 3,79 (1s, 3H, CH_3), 4.14 (1d, 2H, CH_2, J= 6), 5.78 (1d, 1H, NH, J= 7.56), 5.94 (1t, 1H, NH, J= 5.74), 6.89 (1t, 1H, CH_{arom}, J= 7.37), 6.96 (1d, 1H, CH_{arom}, J= 8.13), 7.16 (1d, 1H, CH_{arom}, J= 7.07), 7.21 (1t, 1H, CH_{arom}, J= 7.48, 8.03). Anal. Calculated (%) for $C_{12}H_{18}N_2OS$: C, 60.47 ; H, 7.61 ; N, 11.75; found: C, 60.10; H, 7.64; N, 12.02.

1-(2-Methoxybenzyl)-3-allylthiourea (3c)

White powder (60,65%). mp: 64-66 °C. IR

(KBr 2%, v cm^{-1}):3295, 3180, 1560,1565, 3000, 2840. ^{1}H NMR (DMSO-d_6, 500MHz, δ ppm, J Hz):3.81 (s, 3H, CH_3),4.06 (s, 2H, CH_2), 4.60 (s, 2H, CH_2), 5.08 (d, 1H, CH., J= 10), 5.15 (d, 1H, CH, J= 17), 5.85 (m, 2H, CH), 6.91 (t, 1H, CH, J= 7, 1.5), 6.84 (d, 1H, CH_{arom}, J= 8), 7.17 (d, 1H, CH_{arom}, J= 7), 7.25 (1t, 1H, CH_{arom}, J= 8, 1.5).^{13}C NMR (DMSO-d_6, 125MHz, δ ppm) :44.31 (**C**$\mathbf{H_2}$NH), 55.27 (O**C**H_3), 110.42 (C_{arom}), 115.39 (C_{arom}), 120.06 (C_{arom}), 128.16 (C_{arom}), 131.02 (C_{arom}), 135.13 (C_{arom}), 144.7 (C_{arom}), 156.65 (O**C**$_{arom}$), 178.94 (**C**=S).Anal.Calculated (%) for $C_{12}H_{16}N_2OS$**:** C, 60.98 ; H, 6.82 ; N, 11.85; found: C, 60.70 ; H, 6.80; N, 12.16.

1-(2-Methoxybenzyl)-3-ethylurea (3d)

White powder (80,45 %).Mp: 94-96°C. IR (KBr 2%, v cm^{-1}): v (NH) = 3290, 3180, 3120, 1565,3000, 2840.^{1}H NMR (DMSO-d_6, 500MHz, δ ppm, J Hz):0.99 (t, 3H, NHCH$_2$**C**$\mathbf{H_3}$, J = 7.5Hz), 3.015 (m, 2H, NH**C**$\mathbf{H_2}$CH$_3$, J = 5Hz, 10Hz), 3.79 (s, 3H, OCH_3), 4.155 (d, 2H, CH_2, J = 5Hz), 5.90 (t, 1H, NH, J = 7.5Hz), 6.06 (t, 1H, NH, J = 7.5Hz), 6.89 (t, 1H, CH_{arom}, J = 10Hz), 6.95 (d, 1H, CH_{arom}, J = 10Hz), 7.17 (dd, 1H, CH_{arom}, J = 5Hz, 10Hz), 7.21 (t, 1H, CH_{arom}, J = 10Hz). ^{13}C NMR (DMSO-d_6, 125MHz, δ ppm) :16.15 (CH_3), 34.57 (CH_2), 38.57 (CH_2), 55.70 (OCH_3), 110.82 (C_{arom}), 120.54 (C_{arom}), 128.17 (C_{arom}), 128.26 (C_{arom}), 128.82 (C_{arom}), 157.13 (O**C**$_{arom}$), 158.46 (**C**=O).Anal. Calculated (%) for $C_{11}H_{16}N_2O_2$: C, 63.44; H, 7.74; N, 13.45; Found: C, 63.41.87; H, 7.72; N, 13.40.

1-(2-Ethoxybenzyl)-3-cyclohexylthiourea (3e)

White powder (58,42 %). mp: 142-143 °C. IR (KBr 2%, v cm^{-1}):3300, 3280, 1560, 3000, 2940.^{1}HNMR (DMSO-d_6, 500MHz, δ ppm, J Hz):1.16 (m, 4H, 2CH_2), 1.27 (m, 2H, CH_2); 1.36 (t, 3H, CH_3, J= 7); 6.91(dd, 1H, CH_{arom},),1.54 (m, 1H, CH),1.65(m, 2H,CH_2),1.86 (m, 2H, CH_2),3.99 (brs, 1H, NH), 4.06 (q, 2H, J= 7), 4.59 (s, 2H,CH_2), 6.89 (t, 1H, J= 7.5, 1.5), 6.97 (d, 1H, CH_{arom}, J= 8), 7.17 (d, 1H, CH_{arom}, J= 7), 7.22 (t, 1H, CH_{arom}, J= 7.5, 1.5), 7.41 (brs, 1H, NH). ^{13}C NMR (DMSO-d_6, 125MHz, δ ppm) :14.70 (OCH$_2$**C**H_3), 24.46 (**C**H_2), 25.15 (**C**H_2), 32.30 (**C**H_2), 45.97 (NH**C**H_2), 53.26 (**C**H), 63.22 (O**C**H_2CH_3), 111.38 (C_{arom}), 116.79 (C_{arom}), 116.97 (C_{arom}), 119.99 (C_{arom}), 128.15 (C_{arom}), 155.97 (OC_{arom}), 184.02 (C=S). Anal. Calculated (%) for $C_{16}H_{23}N_2OS$: C, 65.94 ; H, 7.95; N, 9.61;Found: C, 64.64; H, 8.22; N, 9.81.

1-(2-Methoxybenzyl)-3-(4-nitrophenyl) thiourea (3f)

White powder (70,45 %). mp 190 °C.IR (KBr 2%, v cm^{-1}): 3420, 3380,1560,3010, 2880. 1H NMR (DMSO-d_6, 500MHz, δ ppm, J Hz):3.85 (1s, 3H, OCH_3), 4.685 (1d, 2H, CH_2, J= 5), 6.94 (1t, 1H, CH_{arom}, J= 10), 7.03 (1d, 1H, J= 10),7.285 (1m,, 2H, CH_{arom}, 5, 10), 7.89 (1d, 2H, CH_{arom}, J= 10), 8.185 (1d, 2H, CH_{arom}, J= 5), 8.45 (1s, 1H, NH), 10.24 (brs, 1H, NH). ^{13}C NMR (DMSO-d_6, 125MHz, δ ppm) :42.80 (CH_2NH), 55.38 (OCH_3), 110.61 (C_{arom}), 120.13 (C_{arom}), 120.31 (C_{arom}), 124.48 (C_{arom}), 125.32 (C_{arom}), 128.62 (C_{arom}), 128.69 (C_{arom}), 141.75 (C_{arom}), 146.38 (C_{arom}), 156.81 (OC_{arom}), 180.11 (C=S). Anal. Calculated (%) for $C_{15}H_{15}N_3O_3S$:C, 56.77; H, 4.76; N, 13.24;Found:C, 55.78; H, 4.75; N, 13.11.

1-(2-Methoxybenzyl)-3-(3-nitrophenyl)thiourea (3g)

White powder (68, 75 %); mp 122 °C.IR (KBr 2%, v cm^{-1}): 3300, 3285, 1565, 3010, 2870. 1H NMR (DMSO-d_6, 500MHz, δ ppm, J Hz):3.84 (1s, 3H, OCH_3), 4.695 (1d, 2H, CH_2, J= 5), 6.94 (1t, 1H, CH_{arom}, J= 10),7.03 (1d, 1H, CH_{arom}, J= 10), 7.28 (1m, 2H, CH_{arom}, J=10), 7.58 (1t, 1H, CH_{arom}, J= 10), 7.85 (1d, 1H, CH_{arom}), 7.92 (1d, 1H, CH_{arom}, J= 10), 8.28 (1s, 1H, CH_{arom}), 8.69 (1brs, 1H, NH), 10.05 (1brs, 1H, NH).^{13}C NMR (DMSO-d_6, 125MHz, δ ppm) : 42.72 (CH_2NH), 55.37 (OCH_3), 110.57 (C_{arom}), 116.22 (C_{arom}), 117.95 (C_{arom}), 120.14 (C_{arom}), 125.65 (C_{arom}), 128.15 (C_{arom}), 128.50 (C_{arom}), 129.68 (C_{arom}), 138.19 (C_{arom}), 141.04 (C_{arom}), 147.49 (C_{arom}), 156.94 (OC_{arom}), 180.70 (C=S). Anal.Calculated (%) for $C_{15}H_{15}N_3O_3S$: C, 56.77; H, 4.76; N, 10.10.Found: C, 56.05; H, 4.65; N, 13.56.

1-(2-Methoxybenzyl)-3-(4-fluorophenyl) thiourea (3h)

White powder (%).mp:152-154 °C. IR (KBr 2%, v cm^{-1}):3540, 3420,1545, 3025, 2910.^{1}H NMR (DMSO-d_6, 500MHz, δ ppm, J Hz):3.82 (1s, 3H, OCH_3), 4.665 (1d, 2H, CH_2, J=5), 6.93 (1t, 1H, CHarom, J= 10), 7.00 (1d, 1H, CH_{arom}, J= 5), 7.16 (1t, 2H, CH_{arom}, J= 10), 7.225 (1d, 1H, CH_{arom}, J= 5), 7.26 (1t, 1H, CH_{arom}, J= 10), 7.445 (m, 2H, CH_{arom}, J= 5), 7.91 (1brs, 1H, NH), 9.60 (1brs, 1H, NH).^{13}C NMR (DMSO-d_6, 125MHz, δ ppm) :42.79 (CH_2NH), 55.32 (OCH_3), 110.48 (C_{arom}), 115.06 (C_{arom}), 115.24 (C_{arom}), 120.10 (C_{arom}), 125.58 (C_{arom}), 126.13 (C_{arom}), 128.27 (C_{arom}), 135.46 (C_{arom}), 156.73 (OC_{arom}), 157.98 (C_{arom}), 159.90 (C_{arom}), 181.07 (C=S). Anal. Calculated (%) for $C_{15}H_{15}N_2OSF$:C, 62.07; H, 5.17; N, 9.66; Found: C, 62.04; H,4.07; N, 9.92.

1-(2-Methoxybenzyl)-3-(4-cyanophenyl) thiourea (3i)

White powder (85,20 %). mp:186-187 °C. IR (KBr 2%, v cm^{-1}):3300, 3190, 3175, 1565, 3000,2910. ^{1}H NMR (DMSO-d_6, 500MHz, δ ppm, J Hz):3.84 (1s, 3H, CH_3), 4.68 (d, 2H, CH_2, J= 4.5), 6.93 (t, 1H, CH_{arom}, J= 7.5, 7.0),7.03 (d, 1H, CH_{arom}, J= 8),7.28(dd, 2H, CH_{arom}, J= 7.5, 8.5), 7.74 (d, 2H, CH_{arom}, J= 8.5), 7.81 (d, 2H, CH_{arom}, J= 8.5),8.34 (brs, 1H, NH), 10.05 (brs, 1H, NH).Anal. Calculated (%) for $C_{16}H_{15}N_3OS$**:** C, 64.62; H, 5.08; N, 14.13;Found:C, 64.33; H, 5.13; N, 14.35.

1-(2-Methoxybenzyl)-3-phenylthiourea (3j)

White powder (58,76%). mp:130-132°C. IR (KBr 2%, v cm^{-1}):3320, 3110, 1556, 1545, 3010, 2860.^{1}H NMR (DMSO-d_6, 500MHz, δ ppm, J Hz):3.82 (1s, 3H, CH_3), 4.34 (d, 2H, CH_2, J= 4.5), 6.93 (dd, 1H, CH_{arom}),7.24 (td, 1H, CH_{arom}, J= 7.5, 1.5),7.32 (d, 1H,CHarom), 7.28 (dd, 1H, CH_{arom}, J= 7.5, J= 1.5),7.32 (t, 2H, CH_{arom}, J= 2.5, 8.5),7.46 (d, 2H, CH_{arom}, J= 8), 7.92 (brs, 1H, NH), 9.64 (brs, 1H, NH). Anal. Calculated (%) for $C_{15}H_{16}N_2OS$: C, 66.15; H, 5.92; N, 10.29; found: C, 65.99; H, 5.95; N, 10.67.

1-(2-Ethoxybenzyl)-3-phenylthiourea (3k)

White powder (60,35 %). mp:122°C. IR (KBr 2%, v cm^{-1}):3360, 3335, 1535, 3010, 2875.^{1}H NMR (DMSO-d_6, 500MHz, δ ppm, J Hz): 1,37 (1t, 3H, CH_3, J= 6.94), 4,06 (1q, 2H, CH_2, J = 6.94), 4,25 (1d, 2H, CH_2, J= 5.69), 6,35 (1t, 1H, NH, J= 5.73), 6,88 (1m, 1H, CH_{arom}, J= 7.31, 7.40, 7.46), 6,98 (1d, 2H, CH_{arom}, J= 8.41), 7,21 (1t, 3H, CH_{arom}, J= 7.44, 8.40, 2.51), 7,40 (1d, 2H, CH_{arom}, J= 8.01), 8,54 (1s, 1H, NH).Anal. Calculated (%) for $C_{16}H_{18}N_2OS$: C, 67.10; H, 6.33; N, 9.78; Found: C, 67.04; H, 6.28; N, 9.73.

1-(2-Methoxybenzyl)-3-(3-cyanophenyl) thiourea (3l)

White powder (%). mp:126 °C. IR (KBr 2%, v cm^{-1}):3300, 3280, 1560, 3020, 2870. ^{1}H NMR (DMSO-d_6, 500MHz, δ ppm, J Hz):3.84 (1s, 3H, CH_3), 4.68 (d, 2H, CH_2, J= 4.5),6.93 (t, 1H, CH_{arom}, J = 10Hz), 7.02 (d, 1H, CH_{arom}, J = 10Hz), 7.275 (m, 2H, CH_{arom}), 7.515 (m, 2H, CH_{arom}),7.72 (m, 2H, CH_{arom}),8.31 (s, 1H, NH), 10.03 (s, 1H, NH).Anal. Calculated (%) for $C_{16}H_{15}N_3OS$: C, 64.62; H, 5.08; N, 14.13; Found: C, 64.46; H, 5.21; N, 14.08.

1-(4-cyanophenyl)-3-(2,5-dimethoxybenzyl) thiourea (3m)

White powder (0.84 g, 85.71 %).mp: 137.9-139.1 °C.IR (KBr 2%, v cm^{-1}):3366, 3400, 1515, 3060, 2950. ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm, J Hz):3.84 (1s, 3H, CH_3),3.80 (1s, 3H, CH_3), 4.67 (d, 2H, CH_2, J= 4.9), 6.85 (dd, 1H, CHarom, J= 3, 8.70), 6.88 (d, 1H, CH_{arom}, J= 2.90),6.96 (d, 1H,CH_{arom},J= 8.70), 7.76 (m, 2H, CH_{arom}), 7.82 (m, 2H, CHarom),8.35 (brs, 1H, NH), 10.06 (brs, 1H, NH).^{13}C NMR (DMSO-d_6, 100MHz, δ ppm) : 42.69 (**C**H_2NH), 55.59 (O**C**H_3) 55.83 (O**C**H_3), 104.68 (C_{arom}), 111.52 (C_{arom}), 112.18 (C_{arom}), 115.28 (C_{arom}), 119.12 (**C**N) (C_{arom}), 121.21 (C_{arom}), 126.72 (C_{arom}), 132.75 (C_{arom}), 144.26 (C_{arom}), 150.97 (OC_{arom}), 152.99 (OC_{arom}), 180.30 (C=S.Anal. Calculated (%) for $C_{17}H_{17}N_3O_2S$: C, 62.36; H, 5.23, N, 12.83; Found: C, 62.47; H, 5.30; N, 12.98.

1-(3-cyanophenyl)-3-(2,5-dimethoxy benzyl)thiourea (3n)

White powder (69 %). mp: 124.3 °C. IR (KBr 2%, v cm^{-1}):3385, 3350, 1500, 3180, 2985.^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm, J Hz):3.71 (1s, CH_3, 3H),3.80 (1s, 3H, CH_3), 4.67 (d, 2H, CH_2, J=4.8), 6.85 (m, 2H, CH_{arom}), 6.95 (d, 1H, CH_{arom}, J= 8.7), 7.54 (m, 2H, CH_{arom}), 7.76 (dt, 1H, CH_{arom}, J= 2.3, 7.0); 8.09 (s, 1H, CH_{arom}), 8.24 (brs, 1H, NH), 9.89 (brs, 1H, NH). ^{13}C NMR (DMSO-d_6, 100MHz, δ ppm) :42.74, 55.38 55.82 111.10 (C_{arom}), 111.49 (C_{arom}), 112.09 (C_{arom}), 115.14 (C_{arom}), 118.68 5(**C**N), 125.34 (C_{arom}), 126.95 (C_{arom}), 127.25 (C_{arom}), 129.81 (C_{arom}), 140.55 (C_{arom}), 150.92 (OC_{arom}), 153.00 (OC_{arom}), 180.82 (C=S.Anal. Calculated (%) for $C_{17}H_{17}N_3O_2S$: C, 62.36; H, 5.23; N, 12.83; Found: C, 62.15; H, 5.32; N, 12.98.

R/S-1-[1-(5-bromo-2-ethoxyphenyl)ethyl]-3-(4-cyanophenyl)thiourea (4a)

White powder (89.3 %).Mp: 203.7-205 °C. IR (KBr 2%, v cm^{-1}):3399, 3385, 1530, 3175, 2970.^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm, J Hz):1.36(t, 3H,CH_3,J= 7.0),1.43(d, 3H,CH_3,J=6.8),4.1 (q, 2H, CH_2, J= 1.6, 6.9),5.71 (m,1H, CH, J=6.9), 6.97 (d, 1H,CH_{arom},J= 8.4), 7.39 (m, 2H, CH_{arom}), 7.75 (d, 2H, CH_{arom}, J= 8.9), 7.81 (d, 2H, CH_{arom}, J= 8.7), 8.48 (d, 1H, NH, J= 7.9), 9.97 (brs, 1H, NH). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm) : 14.80 (OCH_2**C**H_3), 20.56 (NHCH**C**H_3), 48.35 (NH**C**HCH_3), 63.68 (O**C**H_2CH_3), 104.62 (C_{arom}), 111.90 (C_{arom}), 114.38 (C_{arom}), 119.11 (**C**N), 121.10 (C_{arom}), 128.82 (C_{arom}), 130.49 (C_{arom}), 132.75 (C_{arom}), 134.36 (C_{arom}), 144.29 (C_{arom}), 154.76 (OC_{arom}), 179.27 (C=S.Anal. Calculated (%)for $C_{18}H_{18}BrN_3OS$: C, 53.47; H, 4.49; N, 10.39; Found: C, 53.24; H, 4.48; N, 10.47.

R/S-1-[1-(5-bromo-2-ethoxyphenyl)ethyl]-3-(3-cyanophenyl)thiourea (4b)

White powder (78.8 %). mp: 164.6-165.2 °C. IR (KBr 2%, v cm^{-1}):3385, 1540, 3180, 2990.^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm, J Hz):1.36(t, 3H,CH_3, J=6.9), 1.43(d, 3H,CH_3,J=

7.0),4.1 (m, 2H, CH_2),5.71 (m, 1H, CH, J=5.63), 6.98 (d, 1H,CH_{arom}, J= 9.2), 7.39 (m, 2H, CH_{arom}), 7.53 (m, 2H, CH_{arom}), 7.76 (dt, 1H, CH_{arom}, J=2.2Hz, 6.8Hz),8.09 (s, 1H, CH_{arom}), 8.36 (d, 1H, NH,J=7.7), 9.79 (brs, 1H, NH). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm) : 14.60 (OCH_2**C**H_3), 20.65 ($NHCH$**C**H_3), 48.48(NH**C**HCH_3), 63.68 (O**C**H_2CH_3), 111.07 (C_{arom}), 111.90 (C_{arom}), 114.39 (C_{arom}), 118.65 (**C**N), 125.16 (C_{arom}), 127.14 (C_{arom}), 128.87 (C_{arom}), 129.79 (C_{arom}), 130.47 (C_{arom}), 134.49(C_{arom}), 140.63 (C_{arom}), 154.75 (OC_{arom}), 179.81 (C=S. Anal. Calculated (%) for $C_{18}H_{18}BrN_3OS$: C, 53.47; H, 4.49; N, 10.39; Found: C, 53.72; H, 4.46; N, 10.51.

R/S-1-[1-(2-(benzyloxy)-5-bromophenyl) ethyl]-3-(4-cyanophenyl)thiourea (4c)

White powder (88.15 %). mp: 161.2-162.6°C. IR (KBr 2%, v cm^{-1}):3410, 3275, 1680, 3125, 2970.^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm, J Hz):1.45(d, 3H,CH_3,J=6.8), 5.22 (m, 2H, CH_2),5.81 (m, 1H, CH, J=6.7),7.06 (d, 1H, CHarom, J= 8.7); 7.33 (m, 1H, CHarom); 7.39 (m, 3H, CHarom);7.44 (d, 1H, CH_{arom}, J= 2.05), 7.52 (d, 2H, CH_{arom}, J= 7.3), 7.75 (d, 2H, CH_{arom}, J= 8.7), 7.81 (d, 2H, CH_{arom}, J= 8.5), 8.57 (d, 1H, NH, J= 7.7), 9.96 (brs, NH,1H). ^{13}C NMR (DMSO-d_6, 100MHz, δ ppm) :20.79 ($NHCH$**C**H_3), 48.02 (NH**C**HCH_3), 69.60 (O**C**$H_2C_6H_5$), 104.62 (C_{arom}), 112.35 (C_{arom}), 114.76 (C_{arom}), 119.12 (**C**N), 121.10 (C_{arom}), 127.30 (C_{arom}), 127.80 (C_{arom}), 128.44 (C_{arom}), 128.70 (C_{arom}), 130.39 (C_{arom}), 132.75 (C_{arom}), 134.87 (C_{arom}), 136.81 (C_{arom}), 144.28 (C_{arom}), 154.26 (OC_{arom}), 179.38 (C=S. Anal. Calculated (%) for $C_{23}H_{20}BrN_3OS$: C, 59.23; H, 4.32; N, 9.01; Found: C, 59.60; H, 4.41; N, 9.22.

R/S-1-[1-(2-(benzyloxy)-5-bromophenyl) ethyl]-3-(3-cyanophenyl)thiourea (4d)

White powder (97.4 %). mp: 150.3-151.1 °C. IR (KBr 2%, v cm^{-1}):3295, 3260, 1530, 3125, 2940.^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm, J Hz):1.44(d, 3H, CH_3,J=7.0),5.22 (m, 2H, CH_2),5.83 (m, 1H, CH, J= 6.7),7.06 (d, 1H, CH_{arom}, J= 8.9), 7.33 (m, 1H, CH_{arom}), 7.39 (m, 3H, CH_{arom}), 7.44 (d, 1H, CH_{arom}, J= 2.0), 7.51 (m, 4H, CH_{arom}), 7.76 (d, 1H, CH_{arom}, J= 6.8), 8.09 (s, 1H, CH_{arom}), 8.47 (d, 1H, NH, J= 7.9), 9.78 (brs, 1H, NH). ^{13}C NMR (DMSO-d_6, 100MHz, δ ppm) : 20.88 ($NHCH$**C**H_3), 48.07 (NH**C**HCH_3), 69.60 (O**C**$H_2C_6H_5$), 111.06 (C_{arom}), 112.35 (C_{arom}), 114.76 (C_{arom}), 118.66 (**C**N), 125.20 (C_{arom}), 127.14 (C_{arom}), 127.79 (C_{arom}), 128.44 (C_{arom}), 128.72 (C_{arom}), 129.77 (C_{arom}), 130.37 (C_{arom}), 135.03(C_{arom}), 136.81 (C_{arom}), 140.64 (C_{arom}), 154.25 (OC_{arom}), 179.94 (C=S. Anal. Calculated (%) for $C_{23}H_{20}BrN_3OS$: C, 59.23; H, 4.32; N, 9.01; Found: C, 59.12; H, 4.58; N, 8.83.

R/S-1-[1-(5-chloro-2-ethoxyphenyl)ethyl]-3-(4-cyanophenyl)thiourea (4e)

White powder (67.7 %). mp: 191.7-192.2 °C. IR (KBr 2%, v cm^{-1}):3410, 3385,1530, 3064, 2975. ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm, J Hz):1.36(t, 3H,CH_3,J=6.8),1.43(d, 3H,CH_3,J= 6.8),4.1 (m, 2H, CH_2),5.70 (m, 1H,CH, J= 6.83),7.03 (d, 1H,CH_{arom},J= 8.5), 7.26 (m, 2H, CH_{arom}), 7.75 (d, 2H,CH_{arom},J= 8.7), 7.81 (d, 2H,CH_{arom},J= 8.5), 8.47 (d, 1H,CH_{arom},J= 7.5), 9.96 (brs, 1H, NH). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm) :14.62 (OCH_2**C**H_3), 14.62 (OCH_2**C**H_3), 20.52 ($NHCH$**C**H_3), 48.39 (NH**C**HCH_3), 63.74 (O**C**H_2CH_3), 104.61 (C_{arom}), 113.85 (C_{arom}), 119.12 (**C**N), 121.10 (C_{arom}), 124.09 (C_{arom}), 126.04 (C_{arom}), 127.53 (C_{arom}), 132.74 (C_{arom}), 133.93 (C_{arom}), 144.29 (C_{arom}), 154.32 (OC_{arom}), 179.29 (C=S.Anal. Calculated (%) for $C_{18}H_{18}ClN_3OS$: C, 60.07; H, 5.04; N, 11.68; Found: C, 60.39; H, 5.07; N, 11.88.

R/S-1-[1-(5-chloro-2-ethoxyphenyl)ethyl]-3-(3-cyanophenyl)thiourea (4f)

White powder (72.2 %). mp: 175.3-175.7 °C. IR (KBr 2%, v cm^{-1}):3375, 1540, 3035, 2970.^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm, J Hz):1.36(t, 3H,CH_3, J= 6.9),1.43(d, 3H,CH_3, J=6.8),4.1 (m, CH_2, 2H),5.71(m, 1H, CH, J= 6.5),7.03 (d, 1H, CH_{arom},J= 8.5), 7.27 (m, CH_{arom},2H), 7.53 (m,$_{CHarom}$,2H), 7.75 (dt, 1H, CH_{arom}, J= 2.3, 6.7),8.09(brs, 1H, CH_{arom}), 8.36 (d, 1H, NH, J= 7.9), 9.79 (brs, 1H, NH).^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm) :14.61 (OCH_2**C**H_3), 20.61 ($NHCH$**C**H_3), 48.52 (NH**C**HCH_3), 63.94 (O**C**H_2CH_3), 111.07 (C_{arom}), 113.87 (C_{arom}), 118.65 (**C**N), 124.08 (C_{arom}), 125.16 (C_{arom}), 126.09 (C_{arom}), 127.13 (C_{arom}), 127.51 (C_{arom}), 129.78 (C_{arom}), 134.05 (C_{arom}), 140.63 (C_{arom}), 154.31 (OC_{arom}), 179.82(C=S. Anal. Calculated (%) for $C_{18}H_{18}ClN_3OS$: C, 60.07; H, 5.04; N, 11.68; Found: C, 60.00; H, 4.88; N, 11.70.

R/S-1-[1-(2-ethoxy-5-fluorophenyl)ethyl]-3-(4-cyanophenyl)thiourea (4g)

White powder (54.7 %). mp: 158.6-160.1 °C. IR (KBr 2%, v cm^{-1}):3400, 3230, 1535, 3120, 2970.^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm, J Hz):1.36(t, 3H,CH_3, J=6.8),1.44(d, 3H,CH_3,J= 6.8),4.08 (q, 2H, CH_2, J= 6.5),5.70 (m, 1H, CH, J= 6.5),7.03 (m, 2H, 2.3), 7.1 (dd, 1H, CH_{arom}, J = 2.4,9.4),7.75 (d, 2H, CH_{arom}, J= 8.5), 7.81 (d, 2H, CH_{arom}, J= 8.5), 8.45 (d, 1H, NH, J= 7.3), 9.96 (brs, 1H, NH). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm) : 14.72 (OCH_2**C**H_3), 20.50 ($NHCH$**C**H_3), 48.47 (NH**C**HCH_3), 64.18 (O**C**H_2CH_3), 104.60 (C_{arom}), 113.04 (C_{arom}), 113.28 (C_{arom}), 113.46 (C_{arom}), 113.54 (C_{arom}), 113.60 (C_{arom}), 113.83 (C_{arom}), 119.11 (**C**N)

121.09 (C_{arom}), 132.73 (C_{arom}), 133.74 (C_{arom}), 133.81 (C_{arom}), 144.30 (C_{arom}), 151.77 (C_{arom}), 151.78 (C_{arom}), 155.16 (OC_{arom}), 157.51 (F-C_{arom}), 179.30 (C=S). Anal. Calculated (%) for $C_{18}H_{18}FN_3OS$: C, 62.95; H, 5.28; N, 12.24; Found: C, 63.26; H, 5.24; N, 12.25.

R/S-1-[1-(2-ethoxy-5-fluorophenyl)ethyl]-3-(3-cyanophenyl)thiourea (4h)

White powder (86 %). mp: 162.6-163 °C. IR (KBr 2%, v cm^{-1}):3368, 3165, 1535, 3075, 2990.^{1}HNMR (DMSO-d_6, 400 MHz, δ ppm, J Hz):1.36(t, 3H,CH_3,J= 6.9),1.43(d, 3H,CH_3,J= 7.0),4.08 (m, 2H, CH_2),5.71 (m, 1H, CH, J= 6.7),7.03 (m, 2H, CH_{arom}), 7.1 (dd, 1H, CH_{arom}, 2.9, J= 9.6),7.52 (m, 2H, CH_{arom}, 2.9),7.76 (dt, 1H, CH_{arom}, J= 2,4, 6.7),8.09 (s, 1H, CH_{arom}), 8.33 (d, 1H, NH, J= 7.7); 9.78 (brs, NH, 1H).^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm) : 14.72 ($OCH_2\underline{\mathbf{C}}H_3$), 20.59 ($NHCH\underline{\mathbf{C}}H_3$), 48.60 ($NH\underline{\mathbf{C}}HCH_3$), 64.18 ($O\underline{\mathbf{C}}H_2CH_3$), 111.06 ($C_{arom}$), 113.09 ($C_{arom}$), 113.32 ($C_{arom}$), 113.46 ($C_{arom}$), 113.54 ($C_{arom}$), 113.58 ($C_{arom}$), 113.81 ($C_{arom}$), 118.65 (**C**N) 125.18 (C_{arom}), 127.12 (C_{arom}), 129.78 (C_{arom}), 133.87 (C_{arom}), 133.93 (C_{arom}), 140.64 (C_{arom}), 151.76 (C_{arom}), 151.78(C_{arom}), 155.16(OC_{arom}), 157.51 (F-C_{arom}), 179.84 (C=S). Anal. Calculated (%) for $C_{18}H_{18}FN_3OS$: C, 62.95; H, 5.28; N, 12.24; found: C, 62.86; H, 5.13; N, 12.34.

R/S-1-[1-(2-benzyloxyphenyl)ethyl]-3-(4-cyanophenyl)thiourea (4i)

White powder (81.17 %). mp: 165.1 °C. IR (KBr 2%, v cm^{-1}):3375, 3170, 1540, 3065, 2925. ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm, J Hz):1.47(d, 3H, CH_3,J=6.8),5.22 (1s, 2H, CH_2),5.88 (m, 1H, CH, J= 6,66 Hz),6.97 (t, 1H, CH_{arom}, J= 7.3), 7.09 (d, 1H, CH_{arom}, J= 8.00 Hz), 7.24 (m, 1H, CH_{arom}); 7.33 (m, 2H, CH_{arom}), 7.39 (m, 2H, CH_{arom}), 7.53 (d, 2H, CH_{arom}, J= 7.2), 7.74 (d, 2H, CH_{arom}, J= 8.7), 7.83 (d, 2H, CH_{arom}, J= 8.4), 8.48 (d, 1H, NH,J= 7.7), 9.95 (brs, 1H, NH). ^{13}C NMR (DMSO-d_6, 62.9MHz, δ ppm) :20.89 ($NHCH\underline{\mathbf{C}}H_3$), 48.39 ($NH\underline{\mathbf{C}}HCH_3$), 69.26 ($O\underline{\mathbf{C}}H_2C_6H_5$), 104.55 ($C_{arom}$), 112.46 119.20 (**C**N) 120.71 (C_{arom}), 121.00 (C_{arom}), 126.44 (C_{arom}), 127.28 (C_{arom}), 127.71 (C_{arom}), 128.11 (C_{arom}), 128.44 (C_{arom}), 131.70 (C_{arom}), 132.79 (C_{arom}), 137.24 (C_{arom}), 144.35 (C_{arom}), 155.10 (OC_{arom}), 179.12 (C=S). Anal. Calculated (%) for $C_{23}H_{21}N_3OS$: C, 71.29; H, 5.46; N, 10.84; Found: C, 71.15; H, 5.82; N, 11.04.

R/S-1-[1-(2-benzyloxy)phenyl)ethyl]-3-(3-cyanophenyl)thiourea (4j)

White powder (51.76 %). mp: 97.6-98.7 °C. IR (KBr 2%, v cm^{-1}):3320, 3280, 1525, 3070, 2860. ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm, J Hz):1.47(d, 3H, CH_3,J=7.0), 5.22 (1s, 2H, CH_2), 5.85 (m, 1H, CH, J=5.80), 6.98 (t, 1H, CH_{arom}, J= 7.3), 7.09 (d, 1H, CH_{arom}, J= 7.9), 7.24 (m, 1H, CH_{arom}), 7.33 (t, 2H, CH_{arom}, J= 6.6), 7.39 (t, 2H,CH_{arom},J= 7.5), 7.52 (m, 4H, CH_{arom}), 7.75 (d, 1H, CH_{arom}, J= 6.7), 8.11 (1s, 1H, CH_{arom}), 8.37 (d, 1H, NH, J= 7.7), 9.76 (brs, 1H, NH).^{13}C NMR (DMSO-d_6, 62.9MHz, δ ppm) :21.02 ($NHCH\underline{\mathbf{C}}H_3$), 48.48 ($NH\underline{\mathbf{C}}HCH_3$), 69.28 ($O\underline{\mathbf{C}}H_2C_6H_5$), 111.06 ($C_{arom}$), 112.47 118.77 (**C**N) 120.72 (C_{arom}), 125.11 (C_{arom}), 126.47 (C_{arom}), 127.11 (C_{arom}), 127.30 (C_{arom}), 127.73 (C_{arom}), 128.11 (C_{arom}), 128.47 (C_{arom}), 129.83 (C_{arom}), 131.88 (C_{arom}), 137.27 (C_{arom}), 140.72 (OC_{arom}), 155.11 (OC_{arom}), 179.72 (C=S). Anal. Calculated (%) for $C_{23}H_{21}N_3OS$: C, 71.29; H, 5.46; N, 10.84; Found: C, 71.28; H, 5.62; N, 11.17.

R/S-1-[1-(2-(benzyloxy)-5-methylphenyl)ethyl]-3-(4-cyanophenyl)thiourea (4k)

White powder (68.67 %). mp: 176.9-178.4 °C. IR (KBr 2%, v cm^{-1}):3390, 3175, 3145, 1520, 3070, 2855.^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm, J Hz):1.45(d, 3H, CH_3,J= 6.8 Hz), 2.26 (1s, 3H, CH_3), 5.18 (1s, 2H, CH_2),5.84 (m, 1H,CH, J= 6.3),6.98 (d, 1H, CH_{arom}, J= 8.4 Hz), 7.04 (d, 1H, CH_{arom}, J= 8.4), 7.13 (1s, 1H, CH_{arom}), 7.31 (m, 1H, CH_{arom}), 7.38 (t, 2H, CH_{arom}, J= 7.3), 7.51 (d, 2H, CH_{arom}, J= 7.3), 7.74 (d, 2H, CH_{arom}, J= 8.7), 7.83 (d, 2H, CH_{arom}, J= 8.4), 8.45 (d, 1H, NH,J= 7.7), 9.95 (brs, NH, 1H).^{13}C NMR (DMSO-d_6, 62.9MHz, δ ppm) : 20.40 ($\underline{\mathbf{C}}H_3$), 21.03 ($NHCH\underline{\mathbf{C}}H_3$), 69.37 ($O\underline{\mathbf{C}}H_2C_6H_5$), 104.55 ($C_{arom}$), 112.57 ($C_{arom}$), 119.21 (**C**N) 119.80 (C_{arom}), 121.01 (C_{arom}), 127.07 (C_{arom}), 127.26 (C_{arom}), 127.66 (C_{arom}), 128.32 (C_{arom}), 128.42 (C_{arom}), 129.36 (C_{arom}), 131.43 (C_{arom}), 132.80 (C_{arom}), 137.38 (C_{arom}), 144.37 (C_{arom}), 152.97 (OC_{arom}), 179.05(C=S). Anal. Calculated (%) for $C_{24}H_{23}N_3OS$: C, 71.79; H, 5.77; N, 10.47; Found: C, 71.32; H, 5.45; N, 10.42.

R/S-1-[1-(2-(benzyloxy)-5-methylphenyl) ethyl]-3-(3-cyanophenyl)thiourea (4l)

White powder (79.52 %). mp: 137.9-139.1°C.IR (KBr 2%, v cm^{-1}):3300, 3265, 1525, 3035, 2875.^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm, J Hz):1.45(d, 3H, CH_3, J= 6.8), 2.27 (1s, 3H, CH_3), 5.18 (1s, 2H, CH_2),5.86 (m, 1H, CH, J= 6.0),6.98 (d, 1H, CH_{arom}, J= 8.4), 7.04 (dd, CH_{arom}, 1H, J= 8.2, 1.54), 7.13 (1s, 1H, CHarom), 7.32 (m, 1H, CH_{arom}), 7.38 (t, 2H, CH_{arom}, J= 7.5), 7.51 (m, 4H, CH_{arom}), 7.75(m, 1H, CH_{arom}), 8.11 (s, 1H, CH_{arom}), 8.35 (d, 1H, NH, J= 8.0), 9.76 (brs, 1H, NH).^{13}C NMR (DMSO-d_6, 62.9MHz, δ ppm) :20.40 ($\underline{\mathbf{C}}H_3$), 21.13 ($NHCH\underline{\mathbf{C}}H_3$), 69.37 ($O\underline{\mathbf{C}}H_2C_6H_5$), 111.06 ($C_{arom}$), 112.55 ($C_{arom}$), 118.75 (**C**N) 119.61 (C_{arom}), 125.08 (C_{arom}), 127.08 (C_{arom}), 127.25 (C_{arom}), 127.65 (C_{arom}), 128.28 (C_{arom}),

128.41 (C_{arom}), 129.35 (C_{arom}), 129.81 (C_{arom}), 131.58 (C_{arom}), 137.37 (C_{arom}), 140.71 (C_{arom}), 152.95 (OC_{arom}), 179.61 (C=S). Anal. Calculated(%) for $C_{24}H_{23}N_3OS$: C, 71.79; H, 5.77; N, 10.47; Found: C, 71.54; H, 5.93; N, 10.52.

Biological activity

Myorelaxant effect on rat aortic rings

Experiments were performed on the aorta, collected from adult female Wistar rats (243-382 g) purchased from Janvier Labs (Le Genest-Saint-Isle, France), as previousely described.[21,23] After anesthesia by intraperitoneal injection of pentobarbital (60 mg/kg, i.p.), thoracic aorta was cleared of adhering fat and connective tissue, without removing the endothelium, and cut into transverse rings (2-3mm long). The segments were suspended under 1.5 g tension by means of two steel hooks (one being connected to a tension transducer) in an organ bath containing 10 mL of a Krebs physiological solution (composition in mM: NaCl 118, KCl 5.6, $CaCl_2$ 2.4, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgCl_2$ 1.2, D-glucose 11). The physiological solution was maintained at 37 °C and pH value of pH 7.4, and continuously bubbled with a mixture of O_2-CO_2 (95-5%). Isometric contractions of aortic rings were measured with a force-displacement transducer connected to a PowerLab/8 S with Chart software (AD instruments, Paris, France) for recording and data analysis. Initially stretched at 1.5 g, rings were allowed to equilibrate for 60 min and the Krebs solution was replaced each 15 min. After that, a final mechanical stretch of 1.5 g was applied to the rings which equilibrate for 15 min before starting the experiment. Aorta ring contraction was inducedby replacing the bathing Krebs solution by 30 or 80 mM KCl solution, which depolarizes VSMC membranes and leads to L-type calcium channel opening and extracellular calcium influx, which increases cytosolic free calcium level and provoques cells constriction.

After KCl-induced constriction, the ring tension stabilized and reached a plateau after 15 min, and the tested drugs diluted in dimethylsulfoxide (DMSO) were added to the organ bath in a cumulative manner until maximal relaxation or up to 300 mM, in a 10-90 ml volume range (maximum final concentration of DMSO <1% v/v). Similar experiment was performed in the presence of vehicle (Same DMSO volume), as control. Some experiments were made in the continuous presence of 1 or 10 mM glibenclamide (a K_{ATP} channel blocker) in the bathing medium. The stabilization of the organ response towards KCl, tested drugs and reference compounds, was obtained at least after 15 min, the time needed to obtain steady-state contraction or relaxation (plateau). The relaxation response was expressed as the percentage of decrease in the contractile response to KCl.

Myorelaxant effect on rat trachea rings

Trachea was removed from the same female rats cited above, anaesthetized with sodium pentobarbital (60 mg/kg, i.p.), and carefully cleaned of adhering adipose and connective tissue. Trachea rings (3-4mm long) were suspended in the organ bath (10 mL) and the experiment progressed in the same conditions as those described above for the rat aorta except for the concentration of the contraction inducer (30 mM KCl only).

Stimulation of elastin synthesis in cultured humainvascular smooth muscle cells

Vascular smooth muscle cells (VSMCs) from human aorta (CC-2571) were purchased from Lonza (Levallois, France) and cultured in an adapted medium SmGM-2 Bulletkit™ (CC-3182, Lonza, Levallois, France) supplemented with 5% fetal calf serum (FCS). The cells were used at passages 9 to 11.The cells were seeded in 96-well plates (20000-25000 cells per well) with 500 µl culture medium per well. At sub-confluency, the 500 µl of 5%-FCS-supplemented culture medium was replaced by 500 µl of a 1%-FCS-supplemented culture medium (FCS deprivation limiting cell proliferation) and 5 µl of selected compound solutions (**2j**, **2n**, **3f**, **3g** and **3i**) in DMSO were added to each well (<1% DMSO in the medium). The concentrations of the compound solutions in DMSO were calculated so that the final concentrations of the tested compounds in the culture medium were: 0 (control: DMSO alone added to cells), 20, 50 and 100 µM. 50 µM diazoxide was used as a positive stimulator of elastin production by vascular smooth muscle cells[21,24,25]. After 48h, the extracellular elastin quantities present in each well were measured spectrophotometrically at 450 nm by ELISA: VSMCs were exposed to the primary antibody to elastin (ab21610, Abcam, Paris, France), before application of the secondary antibody coupled to horseradish peroxidase (HRP). This was followed by addition of the substrate of HRP, 3,3',5,5'-tetramethybenzidine (TMB), the reaction being stopped by addition of sulfuric acid. The reaction end-product was then quantified by measuring its absorbance at 450 nm, which is calibrated to the elastin concentration in the well.

RESULTS AND DISCUSSION

Chemistry

The synthesis route used to prepare ring-opened cromakalim analogues, **2a-r**, **3a-n** and **4a-l**, bearing urea or thioureamoities is described in scheme 1. It starts by reacting the commercial ortho-alkoxybenzylamines**1a** and **1b**, or the previousely described amines **1d-l**, with aproppriate alkyl or aryliso(tio)cyanates in dichloromethane, at room temperature[14,21]. The target compounds were isolated with good yields, after 30 minutes stirring, by filtration and removing the solvent under vacuum. The crude product was washed with diethyl ether and recrystallized in ethyl acetate.

Biology

Relaxant activities on rat aorta and trachea rings

The vasorelaxant activities of compounds **2a-r**, **3a-n** and **4a-l** were evaluated on endothelium-intact rat aortaand trachea rings, pre-contracted with a hyperpotassic 30 mMKCl solution. The results obtained from target compounds, in the concentration range of 1-300 mM, and reference drugs (diazoxide, pinacidil, cromakalim, and verapamil), were expressed as EC_{50} values and summarized in Tables 1. Diazoxide, pinacidil and cromakalim were used as reference PCOs while verapamil was used as a reference VGCC blocker.

According to Table 1, it can be observed that ring-opened analogues of cromakalim, bearing N-alkyl urea groups (**2a-i**) showed weak to moderate vasodilator activities, except compound **2j** (EC_{50}=16.51 □M), which was markedly more active than the reference PCO, diazoxide. On the other hand, compound bearing N-aryl (**2m-r**) or N-arylalkyl (**2k**, **2l**) urea groups, were more active than those bearing N-alkyl groups **2a-i**, especialy**2n**, which showed an EC_{50} value of 13.30 □M. These results confirmed our previous preliminary work, which showed that N-aryl groups were more favourable for the vasodilator activity[14]. Taken as a whole, ring-opened analogues of cromakalim, bearing thiourea groups (**3a-n**), were more active than their analogues bearing urea moieties (**2a-r**). Again, it can be observed that compounds with N-arylgroups (**3f-n**) were markedly more active than those with N-alkyl or N-cycloalkyl groups (**3a-e**), especialy compounds **3f**, **3g**, **3i**, **3m** and **3n** which showed EC_{50} values of 14.33, 13.72, 10.41, 10.4, and 10.4 □M respectively. Furthermore, R_1 group being an ethyle was relatively more favourable for the vasodilator activity than the methyle in both the urea and the thiourea series (**2l** *v s* **2k**, **2n** *vs* **2m**, **2r** *vs* **2q**, **3e** *vs* **3a** and **3k** *vs* **3j**). The introduction of a methyle group on the benzylic carbon atom, creating a chiral center and miming the C4 carbon atom of cromakalim, and keeping N-ayles groups, dramatically increased the vasodilator activity of compounds **4a-l** (**4a-c**, **4e-i** and **4k**), especialy**4a** which showed an EC_{50} value of 1.5 □M. The later was markedly more active than diazoxide (~15 fold), but ~11 and ~3 fold less active than cromakalim and pinacidilrespectivelly. It can be observed that X being an electron-withdrawing group like Cl, F, especially Br, was very favorable for the vasodilator activity. The preferable R_1 group was again the ethyle (CH_2CH_3vs $CH_2C_6H_5$) while the R_3 group should bear a cyano group on the 4-position of the aromatic ring (**4a** *vs* **4b**, **4c** *vs* **4d**, **4e** *vs* **4f**, **4g** *vs* **4h**, **4i** *vs* **4j**, **4a** *vs* **4l**).

Some compounds belonging to the three series obtained, namely **2m**, **2n**, **2j**, **3f**, **3g**, **3i**, and **4e**, were selected for further pharmacological investigations in order to identify their mechanism(s) of action. Firstly, the myorelaxant activities of the selected drugs, cromakalim, diazoxide and verapamil were examined on rat aortic rings precontracted by 80 mM KC1. The later concentration strongly inhibits or blocks K_{ATP} channels. In these conditions, the potency of K^+ channel openers should be reduced compared to that exerted against 30 mMKCl induced contractions[27-29],while drugs directly acting on Ca^{2+} channels, such as Ca^{2+} entry blockers (Exemple: verapamil), should maintain the same myorelaxant efficacy on 30 and 80 mMKClprecontracted aortic rings[30]. Indeed, pure potassium channel openers are able to suppress smooth muscle contractions induced by low K^+ concentrations (30mM or less), but not high depolarizing K^+ concentrations (80 mM). At the later concentrations (80 mM), potassium equilibrium potential and cell membrane potential are so close that the hyperpolarization induced by K^+ channel opening is too weak and not able to shift cell membrane potential to the threshold, which closes voltage-operated Ca^{2+} channels[10,31,32]. Indeed, Table 2 showed that the reference compounds cromakalim and diazoxide, two PCOs, were potently inhibited by KCl 80 □M (EC_{50} shifted from 0.13 and 22.4 □M to 190.8 and to beyond 300 □M respectively), while verapamil, a VGCC blocker, maintained the same EC_{50} value (0.06 □M with KCl 30 mM and 0.07 □M with KCl 80 mM). Table 2 also showed that compound **2n** presented the same profile of reference compounds cited above, since its ED_{50} value was shifted from 13.30 to beyond 100 □M, which indicated that it extercted its vasodilator activity mainly throuthg the activation of K_{ATP} channels. On the other hand, the ED_{50} value of

2m, **2j**, **3f**, and **4e** did not significantly change when replacing KCl 30 mM by KCl 80 mM solutions, which meant that they mainly acted as VGCC blockers like their previously analogues bearing N-methylated sulfonylureas and reference compound verapamil[21].

Interstingly, the ED_{50} values of **3g** and **3i** were increased approximatively only by ~2 and ~3 folds respectively (from 13.72 and 10.41 to 25.46 and 33.59 □M respectively), indicating that the vasodilator activity of these two compounds could partialy involve the activation of K_{ATP}. Furtherly, these results were confirmed, at least for **3i** and **4e**, when evaluating their vasodilator activity in the presence of KCl 30 mM and the K_{ATP} channel blocker, glibenclamide (table 3). Indeed, the ED_{50} values of **3i** was increased by ~3 fold (from 10.41 to 87.2□□M) while that of **4e** was, on the contrary, slightly decreased (from 6.20 to 5.2 µM), in the presence of KCl 30 mM and 10 µM glibenclamide solutions, which confirm that **4e** was a VGCC blockers like verapamil.

The most potent vasodilator compound, **4e**, was also investingated on tracheal smooth muscle rings, precontracted by KCl 30 mM. Table 4 clearly indicated that **4e** exerted a strong vasoldilating activity (6.2 µM) on trachea, equaling that on aorta (6.0 µM). This result reveals the lack of tissue-selectivity of **4e**, on the contrary of cromakalim, which was clearly selective of vascular smooth muscle.

Stimulation of elastin synthesis in cultured humainvascular smooth muscle cells

We have evaluated the efficiency of diazoxide and five of the most active vasorelaxant compounds (**2j**, **2n**, **3f**, **3g** and **3i**) in stimulating elastin synthesis by cultured human vascular smooth muscle cells (VSMCs).As shown in Figure 3, diazoxide significantly elevated elastin quantity by 34% at 50 µM. At the concentration of 20 µM, compounds **2j** and **3g** significantly stimulated elastin production by 21% (61% of the effect of 50µM diazoxide) and 28%(≈ 82% of the effect induced by 50 µM diazoxide) compared to the effect of the vehicle alone (DMSO), respectively. The other compounds (**2n**, **3f** and **3i**) were inactive on elastin synthesis at this concentration. At 50 µM and 100 µM, all the compounds were inactive on elastin production, with the exception of compound **3g** which induced a decrease in elastin quantity related to a toxic effect on the cells, as observed by microscopy.As it has been shown in our previous work [21], elastin synthesis is stimulated by both K_{ATP} channel activators like diazoxide, or VGCC blockers like verapamil, this work confirms this finding since that **2j** turned out to be a VGCC blocker while 3g has shown a K_{ATP} channel activating profile.

CONCLUSION

Starting from the K_{ATP} channel opener cromakalim, structural modulations by ring opening and introduction of urea and thiourea moieties, resulted in new compounds, which were investigated on the rat aorta ring model. All compounds were tested for their vasodilatory activity comparatively to the reference compounds, cromakalim, pinacidil and diazoxide (ATP-sensitive potassium channel activators), and verapamil (a voltage-gated calcium channel blocker). Among the best active compounds, one was tested on the rat trachea ring model.

The results obtained on rat aorta rings showed that thiourea derivatives, especially those bearing N-aryl groups, were markedely more active than urea derivatives (**3a-n** and **4a-l** vs **2a-r**, respectively).These results confirmed our previous work which showed that N-aryl groups were more favourable for the vasodilatory activity[14]. The introduction of a methyl group on the benzylic carbon atom, which mimicked the chiral center of cromakalim, dramatically increased the vasodilator activity (**4a-c**, **4e-h**). Indeed, the best active compound was **4a** which showed an ED_{50} value of 1.5 ± 0.4 µM (5), and was almost 15-fold more active than diazoxide but was 4-fold and 11-fold less active than pinacidil and cromakalim, respectively. Furthermore, an ethyl R_1 group being was relatively more favourable for the vasodilator activity than a methyl or benzyl R_1 group in both the urea and thiourea series (**2l***vs***2k**, **2n***vs***2m**, **2r***vs***2q**, **3e***vs***3a**, **3k***vs***3j, 4a *vs* 4c and 4b *vs* 4d**).Also, a cyano group on the 4-position of the aromatic ring as the R_3 group was more favourable for the vasodilatory activity (**4a***vs***4b**, **4c***vs***4d**, **4e***vs***4f**, **4g***vs***4h**, **4i** *vs***4j**, **4k***vs***4l**).

The results also showed that some selected compounds (**2m**, **2n**, **2j**, **3f**, **3g**, **3i**, and **4e**) could be divided into three categories according to their mechanism of action. The first one, represented by **2n**, showed a profile similar to that of diazoxide and cromakalim, which meant that it was a clear K_{ATP} channel activator. The second one, represented by **2m**, **2j**, **3f**, and **4e**, exhibited a VGCC blocker profile similar to that of the reference molecule verapamil and previously developed analogues bearing N-methylated sulfonylureas[21]. The third category was represented by **3g** and **3i**, which could be considered as partial K_{ATP} channel activators. These findings reveal that it is possible to oriente the mechanism of action of these

molecules by varying the nature of substituents on the molecular skeleton.

Further investigation on rat trachea rings revealed that compound **4e** present a vasodilatory effect similar to that on rat aorta rings, but was interestingly found to be 21-fold more active than cromakalim on the trachea, which makes it non-tissue-selective, contrarily to cromakalim which is known to be selective for the vascular smooth muscle.

Investigations on elastin synthesis that **2j** (20µM) increased elastin production by 21%, which represents approximately 61% of the effect of 50µM diazoxide, while **3g** (20µM) increased elastin production by 28%, which is around 82% of the effect induced by 50 µM diazoxide, while at the highest concentrations (50-100 µM) **3g** reduced elastin production.

Taken as a whole, these interesting and encouraging results would deserve to be pursued on a greater number of molecules to enhance activity and tissue selectivity and determination of substituents that control the type of mechanism of action.

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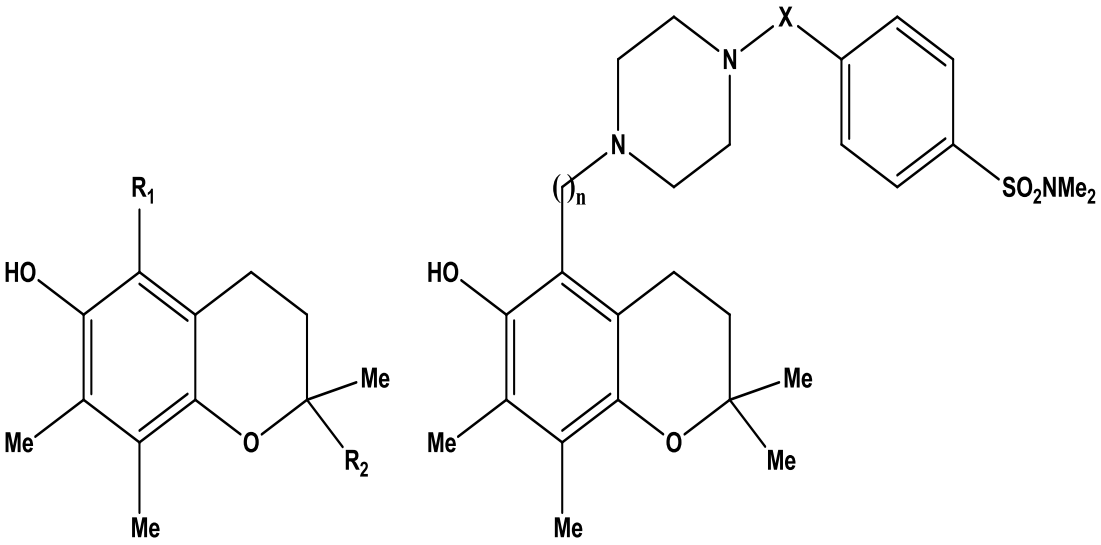


Fig. 1: Some new hybrid compounds combining vitamin E and class I and class III antiarrhythmic drugs[6-9].

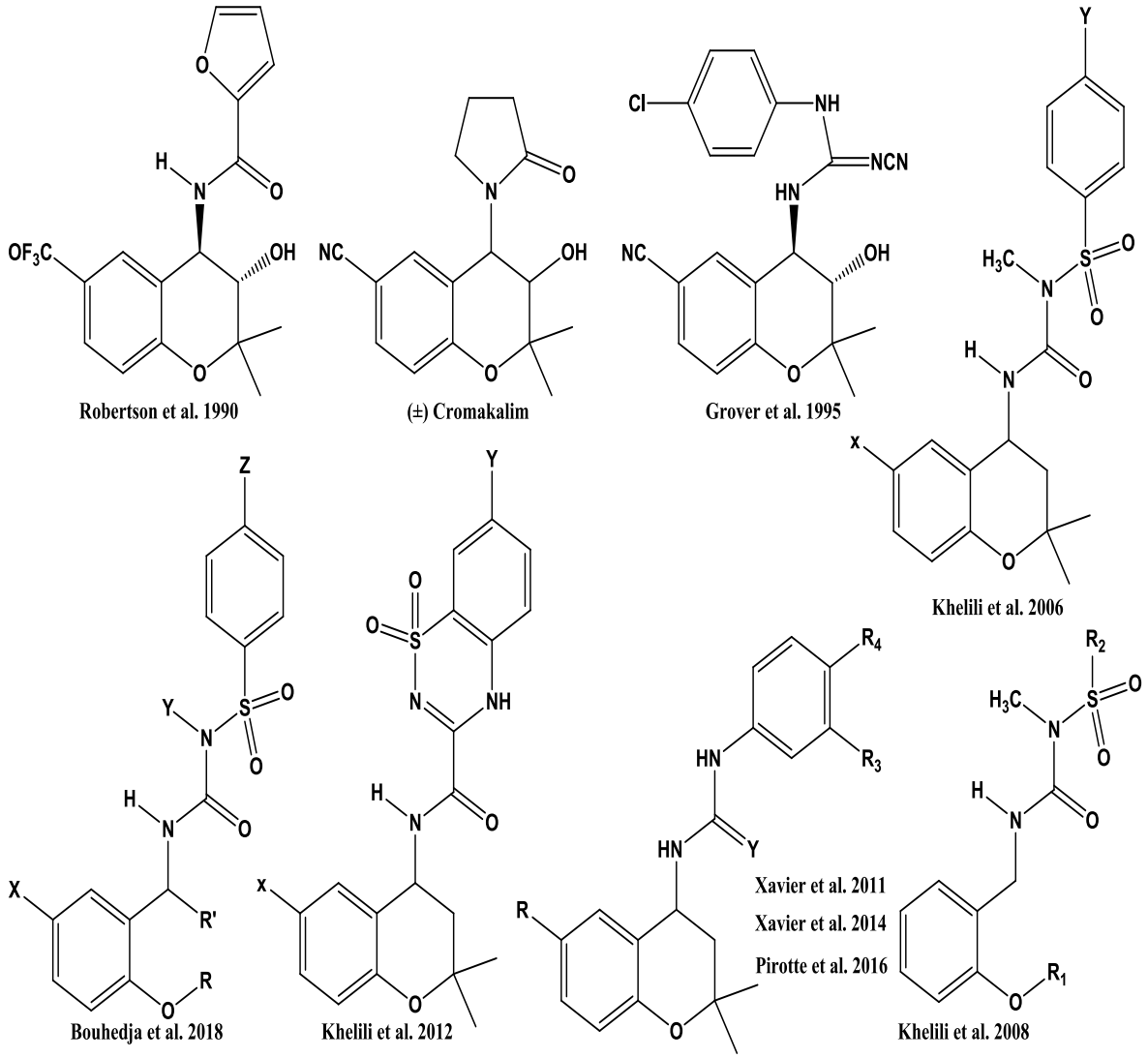


Fig. 2: Chemical structures of cromakalim and some synthetic structural analogues acting on cardiovascular system

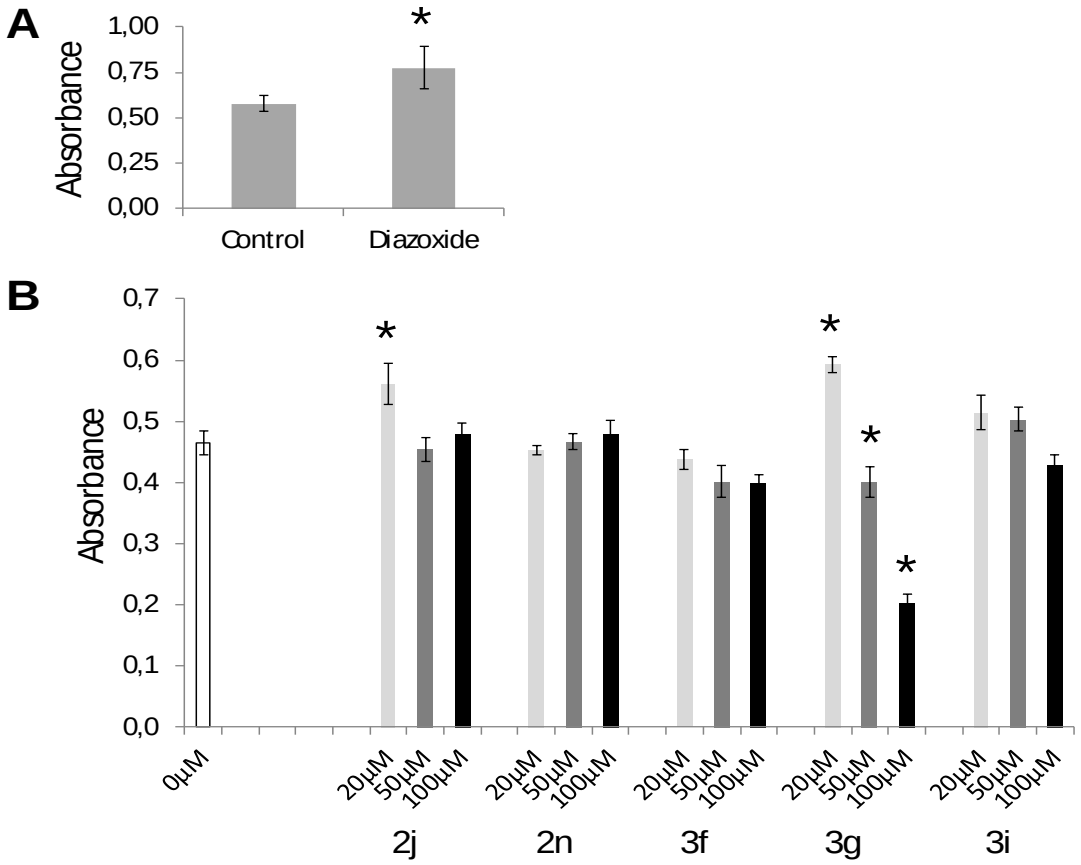
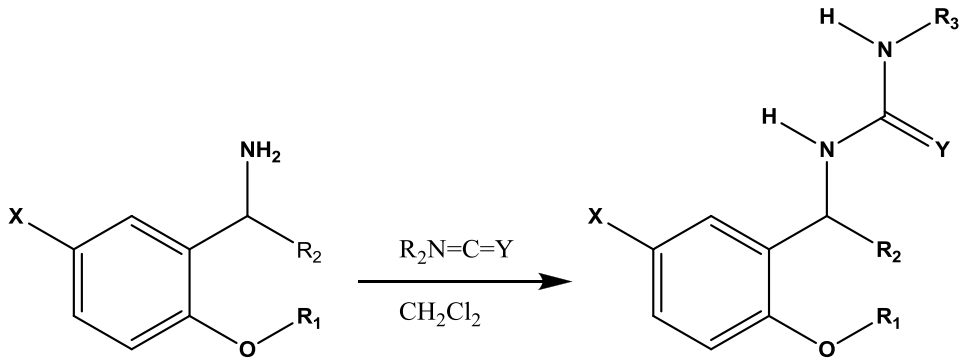


Fig. 3: Effect of selected compounds on elastin production by cultured human CMLVs cells. Absorbance is a function of elastin quantity. The reference stimulator of elastin production diazoxide and the tested molecules were solubilized in DMSO, and their effects were compared to that of DMSO alone (identified as control or 0 µM, in the figure). A: diazoxide was used at the final concentration of 50µM, previously demonstrated to substantially stimulate elastin production in cultured CMLVs (n=3-5 in each group). B: all the other tested compounds (2j, 2n, 3f, 3g, 3i) were used at 3 different concentrations: 20, 50 and 100 µM (n = 4 in each group). *Significant difference with the control (DMSO alone), P ≤ 0.05.



1a-c : X= H, OCH_3, R_1=CH_3, CH_2CH_3, R_2= H
1d-i : X= H, CH_3, F, Cl, Br, R_1= CH_2CH_3, $CH_2C_6H_5$, R_2= CH_3

2a-r : X= H, R_1=CH_3,CH_2CH_3 R_2= H, Y= O, S
3a-n : X= H, OCH_3, R_1=CH_3,CH_2CH_3 R_2= CH_3, Y= S, R_3= alkyl, aryl
4a-l : X= H, CH_3, F, Cl, Br, R_1=CH_3, CH_2CH_3, R_2= CH_3, R_3= aryl, Y= S

Scheme. 1: Synthetic route to target compunds, 2a-r, 3a-n and 4a-l.

Table 1: Effects (EC_{50} (µM)) of compounds 2a-r and 3a-n and 4a-l on the contractile activity of rat aorta rings (Results expressed as means ± SEM (n)).

Compound	X	R_1	R_3	R_2	Y	Vasorelaxant activity EC_{50} (µM)[a]
2a	H	CH_3	$(CH_2)_3CH_3$	H	O	> 30 (9)
2b	H	CH_2CH_3	$(CH_2)_3CH_3$	H	O	> 30 (5)
2c	H	CH_3	$CH(CH_3)_2$	H	O	> 300 (9)
2d	H	CH_2CH_3	$CH(CH_3)_2$	H	O	> 300 (7)
2e	H	CH_3	$C(CH_3)_3$	H	O	> 30 (9)
2f	H	CH_2CH_3	$C(CH_3)_3$	H	O	> 30 (9)
2g	H	CH_3	$CH_2CH=CH_2$	H	O	> 30 (9)
2h	H	CH_2CH_3	$CH_2CH=CH_2$	H	O	> 30 (9)
2i	H	CH_3	$(CH_2)_6CH_3$	H	O	48.06 ± 1.87 (6)
2j	H	CH_3	$(CH_2)_7CH_3$	H	O	16.51 ± 3.63 (5)
2k	H	CH_3	$CH_2C_6H_5$	H	O	60.39 ± 0.98 (5)
2l	H	CH_2CH_3	$CH_2C_6H_5$	H	O	31.12 ± 2.89 (5)
2m	H	CH_3	*o*-$OCH_3C_6H_5$	H	O	36.10 ± 2.58 (5)
2n	H	CH_2CH_3	*o*-$OCH_3C_6H_5$	H	O	13.30 ± 2.30 (7)
2o	H	CH_3	*3,5-di*$OCH_3C_6H_5$	H	O	36.87 ± 3.39 (9)
2p	H	CH_3	*m*-$OCH_3C_6H_5$	H	O	34.70 ± 0.18 (4)
2q	H	CH_3	C_6H_5	H	O	37.44 ± 1.99 (5)
2r	H	CH_2CH_3	C_6H_5	H	O	30.69 ± 2.89 (5)
3a	H	CH_3	C_6H_{11}	H	S	39.09 ± 3.94 (4)
3b	H	CH_3	$CH(CH_3)_2$	H	S	43.25 ± 1.36 (6)
3c	H	CH_2CH_3	$CH_2CH=CH_2$	H	S	48.53 ± 2.00 (6)
3d	H	CH_3	CH_2CH_3	H	O	> 300 (9)
3e	H	CH_2CH_3	C_6H_{11}	H	S	25.27 ± 4.51 (4)
3f	H	CH_3	4-NO_2 C_6H_5	H	S	14.33 ± 3.49 (4)
3g	H	CH_3	3-NO_2 C_6H_5	H	S	13.72 ± 0.95 (4)
3h	H	CH_3	4-F-C_6H_5	H	S	26.93 ± 2.19 (4)
3i	H	CH_3	4-CN-C_6H_5	H	S	10.41 ± 1.86 (4)
3j	H	CH_3	C_6H_5	H	S	35.03 ± 2.07 (4)
3k	H	CH_2CH_3	C_6H_5	H	S	26.90 ± 2.99 (4)
3l	H	CH_3	3-CNC_6H_5	H	S	30.10 ± 2.29 (3)
3m	OCH_3	CH_3	4-CNC_6H_5	H	S	10.4 ± 1.0 (3)
3n	OCH_3	CH_3	3-CNC_6H_5	H	S	10.4 ± 1.1 (3)
4a	Br	CH_2CH_3	4-CNC_6H_5	CH_3	S	01.5 ± 0.4 (5)
4b	Br	CH_2CH_3	3-CNC_6H_5	CH_3	S	10.3 ± 1.0 (4)
4c	Br	$CH_2C_6H_5$	4-CNC_6H_5	CH_3	S	18.4 ± 1.2 (3)
4d	Br	$CH_2C_6H_5$	3-CNC_6H_5	CH_3	S	55.6 ± 4.8 (4)
4e	Cl	CH_2CH_3	4-CNC_6H_5	CH_3	S	06.2 ± 1.2 (5)
4f	Cl	CH_2CH_3	3-CNC_6H_5	CH_3	S	10.9 ± 1.3(4)
4g	F	CH_2CH_3	4-CNC_6H_5	CH_3	S	08.7 ± 1.1 (3)
4h	F	CH_2CH_3	3-CNC_6H_5	CH_3	S	14.0 ± 2.0 (4)
4i	H	$CH_2C_6H_5$	4-CNC_6H_5	CH_3	S	19.4 ±1.5 (3)
4j	H	$CH_2C_6H_5$	3-CNC_6H_5	CH_3	S	63.2 ± 3.9 (4)
4k	CH_3	$CH_2C_6H_5$	4-CNC_6H_5	CH_3	S	17.2 ± 2.1 (3)
4l	CH_3	$CH_2C_6H_5$	3-CNC_6H_5	CH_3	S	28.8 ± 3.4 (4)
Cromakalim	-	-	-	-	-	0.13 ± 0.05 (4)
Pinacidil	-	-	-	-	-	0.35 ± 0.02 (11)[b]
Diazoxide	-	-	-	-	-	22.4 ± 2.1 (6)[b]

[a]EC_{50}: drug concentration giving 50% relaxation of the 30 mMKCl-induced contraction of rat aorta rings (mean± SEM (n)). n refers to the number of samples. [b]Published results[26]

Table 2: Myorelaxant effect of selected compounds (2m, 2n, 2j, 3f, 3g, 3i, and 4e) on 30 mMKCl-precontracted rat aorta rings as well as on 80 mMKClprecontracted rat aorta rings

Compound	KCl 30 mM EC_{50} (µM) [a]	KCl 80 mM EC_{50} (µM) [a]
2m	36.10 ± 2.58 (5)	> 30 (6)
2n	13.30 ± 2.30 (7)	>100 (9)
2j	16.51 ± 3.63 (5)	19.01 ± 1.37 (7)
3f	14.33 ± 3.49 (4)	14.68 ± 0.19 (7)
3g	13.72 ± 0.95 (4)	25.46 ± 1.91 (6)
3i	10.41 ± 1.86 (4)	33.59 ± 1.39 (3)
4e	06.20 ± 1.2 (5)	7.60 ± 0.6 (4)

Cromakalim	0.13 ± 0.05 (4)[b]	190.8 ± 39.3 (7)[b]
Vérapamil	0.06 ± 0.02 (4)[c]	0.07 ± 0.02 (4)[c]
Diazoxide	22.4 ± 2.1 (6) [d]	>300 (6)[d]

[a]Results are expressed as (mean± SEM (n)); n number in parentheses refers to the number of samples. [b]Published results[21].[c]Published results[29].[d] Published results[26].

Table 3: Myorelaxant effects of active compound 3i, 4e and cromakalim on 30- and 80-mM induced contraction of rat aorta rings incubated in the absence or the presence of 1 and 10 µM glibenclamide

Compound	Myorelaxant activity 30 mMKCl EC_{50} (µM) [a]		
	0 µM Glib	1 µM Glib	10 µM Glib
3i	10.41 ± 1.86 (4)	15.67 ± 2.49	28.54 ± 2.66
4e	06.20 ± 1.2 (5)	6.3 ± 1.4 (4)	5.2±1.6 (4)
± Cromakalim	0.13 ± 0.05 (4)	3.4 ± 0.8 (5)	87.2±10.5 (4)
Vérapamil	0.06 ± 0.02 (4)[b]	0.05 ± 0.02 (4)[b]	0.07± 0.02 (4)[b]
Diazoxide	22.4 ± 2.1 (6)[b]	85.8 ± 22.2 (6)[c]	163.4 ± 41.2 (6)[c]

[a] Results are expressed as (mean± SEM (n)); n number in parentheses refers to the number of samples. [b]Published results[29]. [c]Published results[26].

Table 4: Effects of 4e and cromakalimon the contractile activity of 30 mM K^+-depolarized rat aorta and rat trachea rings

Compound	Relaxation of precontracted		Selectivity [b]
	rat aorta EC_{50} (µM) [a]	rat trachea EC_{50} (µM)	trachea/aorta
4e	06.2 ± 1.2 (5)	6.0 ± 1.9 (4)	1.0
± Cromakalim	0.13 ± 0.05 (4)	124.4 ± 28.7 (3)	956.9

[a] EC_{50} is the drug concentration reducing by 50% the rat aorta and the rat trachea tonusinduced by 30mM KCl. [b] Selectivity is the ratio of the EC_{50} determined on rat trachea and rat aorta.

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