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Triazole incorporated pyridazinones as a new class of antihypertensive agents: Design, synthesis and in vivo screening

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ABSTRACT

A number of 6-(substituted phenyl)-2-(4-substituted phenyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)-4,5-dihydropyridazin-3(2*H*)-one derivatives were designed and synthesized by a sequence of reactions starting from respective aryl hydrocarbons. The final compounds (**4a**–**4u**) were evaluated for antihypertensive activities by non-invasive method using Tail Cuff method. The compounds **4e**, **4i** and **4k** showed appreciable antihypertensive activity comparable with that of standard hydralazine and propranolol.

The chemistry of pyridazinones has been an interesting field of study since decade. The synthesis of novel pyridazinone derivatives and investigation of their chemical and biological behavior have gained more importance in recent decades for biological, medicinal, and agricultural reason. Living organism finds difficulty in construction of N–N bonds that limits the natural abundance of compounds having such bonds. The pharmacological activity of 4,5-dihydro-6-phenyl-3(2*H*)-pyridazinones has been extensively studied and is known for its cardiovascular effects.[1–3] In this field several compounds such as zardaverine or imazodan have been developed as PDE III inhibitors in the search for new antiplatelet or cardiotonic agents.[4,5]

A survey of literature revealed that substituted pyridazinones have received much attention during recent years on account of their prominent potential as antidepressant,[6] antihypertensive,[7–9] antithrombotic,[10] anticonvulsant,[11] cardiotonic,[12] antibacterial,[13] diuretics,[14] antiHIV,[15] and anticancer.[16] Pyridazinone causes direct relaxation of arteriolar smooth muscle by reducing arterial tone without affecting autonomic nervous system. The molecular mechanisms mediating this action are not clear, but may ultimately involve a fall in intracellular calcium concentrations. While a variety of changes in cellular signaling pathways are influenced by arteriolar vasodilators like hydralazine, precise molecular targets that explain its capacity to dilate arteries remain uncertain. The current work describes the synthesis of novel 4,5-dihydro-3(2*H*)-pyridazinone moiety with encouraging antihypertensive activity by non-invasive method using Tail Cuff method.

The synthesis of novel pyridazinone derivatives has been carried out according to the steps shown in Figure 1. In the initial step, β-aroyl propionic acids (**1a**–**1g**) were synthesized by Friedel–Crafts acylation of appropriate hydrocarbons with succinic anhydride in the presence of anhydrous aluminum chloride.[17] The intermediates (**2a**–**2g**) were synthesized by reacting β-aroyl propionic acids with carbohydrazide in absolute ethanol.[18] The thiosemicarbazides (**3a**–**3u**) conveniently synthesized by refluxing carbohydrazide derivatives (**2a**–**2g**) with aryl isothiocyanate in ethanol. Intermediate thiosemicarbazide (0.05 M) was refluxed in 2 M sodium hydroxide solution (reaction time varies from 4 to 5 h), cooled, and poured into excess of water containing crushed ice. Acidification with glacial acetic acid yielded a solid which was collected by filtration and crystallized from ethanol to give final compounds (**4a**–**4u**).[19] Purity of compounds was checked by single-spot TLC. Two different solvent systems: toluene:ethyl acetate:formic acid (5:4:1) and petroleum ether:toluene:acetic acid (5:4:1), were used to run the TLC and spots were located under iodine vapors/UV light. Compounds were characterized on the basis of spectral data (IR, ^{1}H NMR, mass and elemental analysis). Spectral data of all the newly synthesized compounds were in full agreement with proposed structures.[20] In general, Infra Red Spectra (IR) revealed NH, CH, C=S, C=O and C=N peak at 3323, 2926, 2368, 1685 and 1607 cm^{-1}, respectively. In the Nuclear Magnetic Resonance Spectra (^{1}H NMR) the signals of the respective protons of the prepared titled compounds were verified on the basis of their chemical shifts, multiplicities, and coupling constants. The

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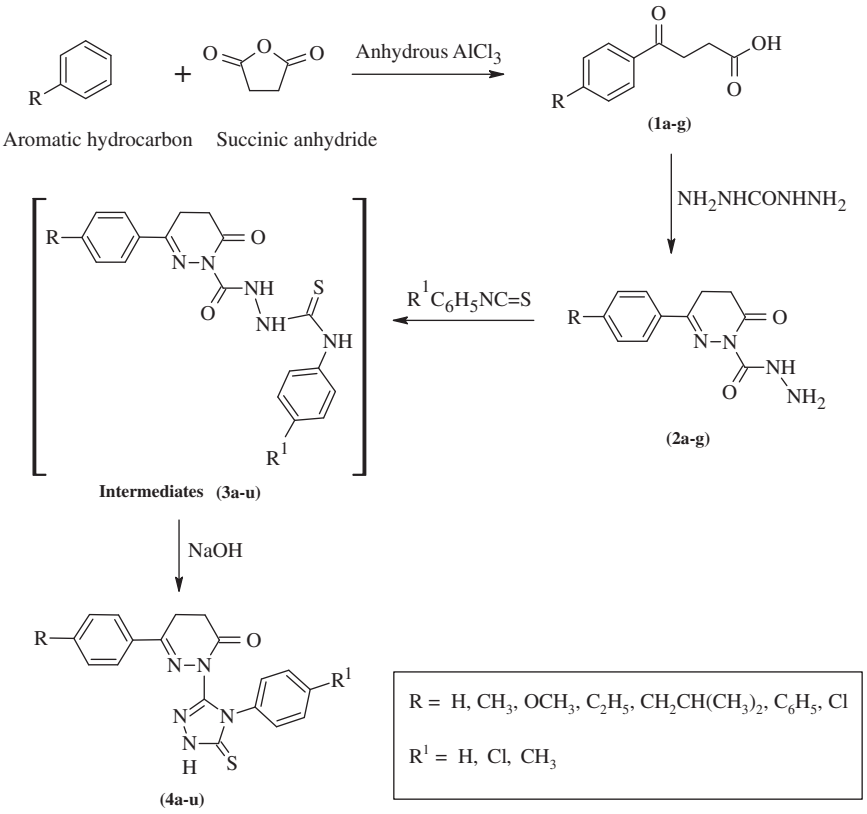

Figure 1. Synthesis of 6-(substituted phenyl)-2-(4-substituted phenyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)-4,5-dihydropyridazin-3(2*H*)-one derivatives.

two triplets at about δ 2.54, J = 7.8 and 2.99, J = 7.8 confirmed the presence of methylene group at 4- and 5-position of pyridazinone ring, respectively. For the compounds **4m**, **4n**, and **4o** the mentioned peaks were shifted at about δ 2.9 and 3.3 ppm, respectively. The multiplets at δ 7.13–7.93 are indicative of aromatic protons. The singlet at δ 10.78 is due to CSNH group flanked by two nitrogen atoms. The mass spectra show the presence of peak at definite m/z value in accordance to the molecular formula. The elemental analysis results were within ±0.4% deviation from the theoretical values.

The final compounds (**4a**–**4u**) were evaluated for antihypertensive activity by non-invasive method using Tail Cuff method. The results were shown in Table 1 and compared with standard drug, hydralazine and propanolol. Compounds **4e**, **4i**, and **4k** were found to show highly significant reduction in mean arterial blood pressure but at higher dose in comparison to standard drugs.[21]

Albino rats (body weight 200–250 g) were supplied by Central Animal House Facility, Hamdard University and kept under standard laboratory conditions in 12 h light/dark cycle at 25 ± 2 °C. Animals were provided with pellet diet (Lipton, Calcutta, India) and water ad libitum. They were marked for easy identification. All the experimental protocols were carried out with the permission from Institutional Animal Ethics Committee (IAEC) and the guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments in Animal (CPCSEA). Animals were obtained from Central Animal House Facility, Hamdard University, New Delhi 62. Registration number and date of registration is 173/CPCSEA, 28 January, 2000.

For conducting the BP measurement studies, the animals were kept in a restrainer for 10 min every day for 1 week. This exercise was done to avoid the fluctuation in blood pressure due to aggressive behavior of animal while keeping into the restrainer for measuring the activity.

After recording the initial BP of rats, the animals were divided into groups of five animals each. One group was taken as control. Hypertension was induced in the remaining groups by subcutaneous injection of methyl prednisolone acetate (20 mg/kg/wk) for 2 weeks.[22]

Mean arterial blood pressure was measured in conscious rats using CODA Non-Invasive Blood Pressure Recorder by Tail–Cuff method (Kent Scientific Corporation, USA). The restrainer carrying the rat was placed in the BP instrument with tail protruding out. The tail was gently placed in contact with a transducer membrane, which was connected to the digital BP display panel. The instrument was then turned on and allowed to stabilize until steady pulse rate was observed. Once the 'pulse level ready' signal appeared, the BP recording button was pressed and the mean arterial BP was recorded. Albino rats (body weight 200–250 g) were used in present study. Rats were assigned to groups of five animals in each. Each compound (20 mg/kg body weight) was injected intraperitoneally after suspending in 1% carboxymethyl cellulose (CMC) solution. The mean arterial blood pressure was recorded after 1 h.

The statistical analysis was performed using GRAPHPAD INSTAT 3 software (Graph Pad Software Inc., San Diego, CA). Data obtained from animal experiments were expressed as arithmetic mean ± SEM. The comparison between various groups was performed by one-way analysis of variance (ANOVA), and the effect in treatment groups were compared with toxic control group by Dunnet multiple comparison test. p <0.05 was considered to be significant ($^{*}p$ <0.05; $^{**}p$ <0.01). The percentage reduction in MABP for all the treatment groups was also calculated and compared.

It can be observed on the basis of structure–activity relationships that groups like p-CH_3, p-OCH_3 and p-C_2H_5 in phenyl ring at 6-position increases the activity as shown by the compound **4e**, **4i** and **4k**. All compounds possessing substituted aryl moiety

Table 1
Mean arterial blood pressure (mm Hg) and substituents of compounds (**4a**–**4u**)

Compd (20 mg/kg)	MABP (mean ± SEM)	% Reduction in MABP	R	R^1
Control	101.33 ± 4.64			
Toxic control	162.33 ± 4.02**			
Propranolol[a]	95.12 ± 4.68**	41.40		
Hydralazine[b]	96.16 ± 4.70**	41.76		
4a	113.6 ± 7.78**	30.01	H	H
4b	122 ± 4.85**	24.84	H	Cl
4c	107.4 ± 5.54**	38.83	H	CH_3
4d	118 ± 7.56**	27.30	CH_3	H
4e	94.41 ± 7.32**	41.84	CH_3	Cl
4f	111.6 ± 10.28**	31.25	CH_3	CH_3
4g	109.6 ± 6.17**	32.48	OCH_3	H
4h	136.2 ± 2.9**	16.09	OCH_3	Cl
4i	95.8 ± 3.93**	40.98	OCH_3	CH_3
4j	99.2 ± ± 2.57**	38.88	C_2H_5	H
4k	93.96 ± 6.61**	42.11	C_2H_5	Cl
4l	108.8 ± 5.02**	32.97	C_2H_5	CH_3
4m	112.8 ± 3.92**	30.51	$CH_2CH(CH_3)_2$	H
4n	136.6 ± 1.75**	15.85	$CH_2CH(CH_3)_2$	Cl
4o	142 ± 4.19*	12.50	$CH_2CH(CH_3)_2$	CH_3
4p	145 ± 3.61**	10.67	C_6H_5	H
4q	139 ± 4.27**	14.37	C_6H_5	Cl
4r	141 ± 3.10**	13.13	C_6H_5	CH_3
4s	142 ± 3.98**	12.52	Cl	H
4t	144 ± 4.41**	11.29	Cl	Cl
4u	143.6 ± 4.13**	11.53	Cl	CH_3

All values were expressed as mean ± SEM ($^*p \leqslant 0.05$), each group comprised of four animals (i.e., $n = 4$).
Toxic control group was compared with control group. All the treatment groups were compared with toxic control group and $p < 0.05$ was considered to be significant.
* $p < 0.05$.
[a] Dose of propanolol was taken as 14 mg/kg.
[b] Dose of hydralazine was taken as 2.6 mg/kg.
** $p < 0.01$.

at C-6 of pyridazin-3(2*H*)-one ring showed significant percent reduction in comparison to the compounds possessing unsubstituted aryl ring. It indicated that substitution on aromatic ring is essential for antihypertensive activity. Substitution with methyl, methoxy and ethyl group at *para* position of phenyl ring on pyridazinone moiety increases the antihypertensive activity significantly. The compounds substituted with group like isobutyl, biphenyl, chloro at *para* position of phenyl ring on pyridazinone moiety did not show appreciable activity.

In summary various 6-(substituted phenyl)-2-(4-substituted phenyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)-4,5-dihydropyridazin-3(2*H*)-one derivatives were prepared. Among these compounds 6-(4-methylphenyl)-2-[4-(4-chlorophenyl)-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl]-4,5-dihydropyridazin-3(2*H*)-one (**4e**), 6-(4-methoxyphenyl)-2-[4-(4-methylphenyl)-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl]-4,5-dihydropyridazin-3(2*H*)-one (**4i**) and 6-(4-ethylphenyl)-2-[4-(4-chlorophenyl)-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl]-4,5-dihydropyridazin-3(2*H*)-one (**4k**) showed maximum antihypertensive activity.

Therefore, it was concluded that triazole incorporated 4,5-dihydro-3(2*H*)-pyridazinone derivatives can be further modified to exhibit better potency than the standard drugs. Further studies to acquire more information about Quantitative Structure Activity Relationships (QSAR) are in progress in our laboratory. The 4,5-dihydro-3(2*H*)-pyridazinone derivatives discovered in this study may provide valuable therapeutic intervention for the treatment of hypertension.

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19. General method for the synthesis of 6-(substituted phenyl)-2-(4-substituted phenyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)-4,5-dihydropyridazin-3(2*H*)-one derivatives (**4a**–**4u**): A ethanolic solution of 6-oxo-3-substituted phenyl-5,6-dihydropyridazine-1(4*H*)-carbohydrazide (0.01 mol) and aromatic isothiocyanate (0.01 mol) was refluxed for 4 h. The contents were concentrated and poured into crushed ice, filtered and dried to crude thiosemicarbazide intermediates as 6-substituted phenyl-2-(*N*-phenylthiosemicarbazido)-4,5-dihydropyridazin-3(2*H*)-one derivatives (**3a**–**3u**). Crude thiosemicarbazide intermediates (0.005 mol) was refluxed in 2 M sodium hydroxide solution (20 ml) for 4–5 h, cooled, poured into excess of water containing crushed ice with continuous stirring and filtered to get the final compound. The filtrate on acidification with glacial acetic acid yielded the precipitate, which was collected by filtration. The combined precipitates were recrystallized from ethanol.

20. Physical and analytical data of the compounds: *Compound* ***4a****:* Yield: 40%; mp 180–182 °C; R_f 0.42; IR (KBr) υ_{max} (cm^{-1}): 3323 (NH), 2930 (CH), 2368 (C=S), 1685 (C=O), 1607 (C=N), 1030; ^1H NMR (300 MHz, $CDCl_3$): δ 2.54 (t, J = 7.8, 2H, C–CH_2), 2.99 (t, J = 7.8, 2H, CH_2–CO), 7.13–7.93 (m, 10H, Ar–H), 10.78 (s, 1H, CSNH); Mass (m/z): 349/350 (M^+/M^++1); Anal. Calcd for $C_{18}H_{15}N_5OS$: C, 61.87; H, 4.33; N, 20.04. Found: C, 61.82; H, 4.22; N, 19.96.
Compound ***4b****:* Yield: 46%; mp 148–150 °C; R_f 0.46; IR (KBr) υ_{max} (cm^{-1}): 3330 (NH), 2922 (CH), 2365 (C=S), 1665 (C=O), 1612 (C=N), 1025, 817; ^1H NMR (300 MHz, $CDCl_3$): δ 2.48 (t, J = 7.7, 2H, C–CH_2), 2.99 (t, J = 7.7, 2H, CH_2–CO), 7.13–7.93 (m, 9H, Ar–H), 10.82 (s, 1H, CSNH); Mass (m/z): 383 (M^+); Anal. Calcd for $C_{18}H_{14}ClN_5OS$: C, 56.32; H, 3.68; N, 18.24. Found: C, 56.28; H, 3.56; N, 18.22.
Compound ***4c****:* Yield: 60%; mp 188–190 °C; R_f 0.46; IR (KBr) υ_{max} (cm^{-1}): 3452 (NH), 2926 (CH), 2360 (C=S), 1648 (C=O), 1600 (C=N); ^1H NMR (300 MHz, $CDCl_3$): δ 2.40 (s, 3H, CH_3), 2.62 (t, J = 7.8, 2H, C–CH_2), 3.2 (t, J = 7.8, 2H, CH_2–CO), 7.2–7.80 (m, 9H, Ar–H), 10.76 (s, 1H, CSNH); Mass (m/z): 363/364 (M^+/M^++1); Anal. Calcd for $C_{19}H_{17}N_5OS$: C, 62.79; H, 4.71; N, 19.27. Found: C, 62.78; H, 4.66; N, 19.02.
Compound ***4d****:* Yield: 40%; mp 192–194 °C; R_f 0.54; IR (KBr) υ_{max} (cm^{-1}): 3426 (NH), 3094 (CH), 2365 (C=S), 1658 (C=O), 1612 (C=N), 809, 699; ^1H NMR (300 MHz, $CDCl_3$): δ 2.30 (s, 3H, CH_3), 2.44 (t, J = 7.6, 2H, C–CH_2), 2.96 (t, J = 7.6, 2H, CH_2–CO), 7.21–7.23 (dd, 2H, J = 7.8, H-3′, H-5′), 7.34–7.40 (m, 5H, Ar–H), 7.61–7.64 (dd, 2H, J = 7.8, H-2′, H-6′), 10.82 (s, 1H, CSNH); Mass (m/z): 363/364 (M^+/M^++1); Anal. Calcd for $C_{19}H_{17}N_5OS$: C, 62.79; H, 4.71; N, 19.27. Found: C, 62.74; H, 4.62; N, 19.16.
Compound ***4e****:* Yield: 38%; mp 196–198 °C; R_f 0.60; IR (KBr) υ_{max} (cm^{-1}): 3215 (NH), 2929 (CH), 2364 (C=S), 1682 (C=O), 1615 (C=N), 760; ^1H NMR (300 MHz, $CDCl_3$): δ 2.34 (s, 1H, CH_3), 2.48 (t, 2H, CH_2), 2.96 (t, J = 7.8, 2H, C–CH_2), 3.20 (t, J = 7.8, 2H, CH_2–CO), 7.13–7.93 (m, 8H, Ar–H), 10.74 (s, 1H, CSNH); Mass (m/z): 397 (M^+); Anal. Calcd for $C_{19}H_{16}ClN_5OS$: C, 57.35; H, 4.05; N, 17.60. Found: C, 57.25; H, 3.96; N, 17.52.
Compound ***4f****:* Yield: 72%; mp 202–204 °C; R_f 0.48; IR (KBr) υ_{max} (cm^{-1}): 3450 (NH), 2924 (CH), 2352 (C=S), 1662 (C=O), 1602 (C=N); ^1H NMR (300 MHz, $CDCl_3$): δ 2.30 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 2.52 (t, J = 7.7, 2H, C–CH_2), 2.96 (t, J = 7.7, 2H, CH_2–CO), 7.21–7.23 (dd, 2H, J = 7.8, H-3′, H-5′), 7.34–7.40 (m, 4H, Ar–H), 7.61–7.64 (dd, 2H, J = 7.8, H-2′, H-6′), 10.84 (s, 1H, CSNH); Mass (m/z): 377/378 (M^+/M^++1); Anal. Calcd for $C_{20}H_{19}N_5OS$: C, 63.64; H, 5.07; N, 18.55. Found: C, 63.58; H, 4.88; N, 18.46.
Compound ***4g****:* Yield: 42%; mp 170–172 °C, R_f 0.56; IR (KBr) υ_{max} (cm^{-1}): 3471 (NH), 2926 (CH), 2374 (C=S), 1647 (C=O), 1612 (C=N), 1092, 794; ^1H NMR (300 MHz, $CDCl_3$): δ 2.58 (t, J = 7.8, 2H, C–CH_2), 2.96 (t, J = 7.8, 2H, CH_2–CO), 3.67 (s, 3H, CH_3O), 7.0–7.94 (m, 9H, Ar–H), 10.86 (s, 1H, CSNH); Mass (m/z): 379/390 (M^+/M^++1); Anal. Calcd for $C_{19}H_{17}N_5O_2S$: C, 60.14; H, 4.52; N, 18.46. Found: C, 60.02; H, 4.48; N, 18.40.
Compound ***4h****:* Yield: 38%; mp 178–180 °C; R_f 0.58; IR (KBr) υ_{max} (cm^{-1}): 3499 (NH), 2920 (CH), 2360 (C=S), 1638 (C=O), 1562 (C=N), 801; ^1H NMR (300 MHz, $CDCl_3$): δ 2.62 (t, J = 7.6, 2H, C–CH_2), 2.94 (t, J = 7.6, 2H, CH_2–CO), 3.82 (s, 3H, CH_3O), 7.12–7.96 (m, 8H, Ar–H), 10.72 (s, 1H, CSNH); Mass (m/z): 413 (M^+); Anal. Calcd for $C_{19}H_{16}ClN_5O_2S$: C, 55.14; H, 3.90; N, 16.92. Found: C, 55.02; H, 3.84; N, 16.84.
Compound ***4i****:* Yield: 52%; mp 182–184 °C; R_f 0.54; IR (KBr) υ_{max} (cm^{-1}): 3458 (NH), 2940 (CH), 2372 (C=S), 1672 (C=O), 1618 (C=N); ^1H NMR (300 MHz, $CDCl_3$): δ 2.40 (s, 3H, CH_3), 2.56 (t, J = 7.6, 2H, C–CH_2), 2.96 (t, J = 7.6, 2H, CH_2–CO), 3.67 (s, 1H, CH_3O), 7.0–7.94 (m, 8H, Ar–H), 10.84 (s, 1H, CSNH); Mass (m/z): 393/394 (M^+/M^++1); Anal. Calcd for $C_{20}H_{19}N_5O_2S$: C, 61.05; H, 4.87; N, 17.80. Found: C, 60.98; H, 4.94; N, 17.64.
Compound ***4j****:* Yield: 46%; mp 196–198 °C; R_f 0.62; IR (KBr) υ_{max} (cm^{-1}): 3424 (NH), 3096 (CH), 2362 (C=S), 1660 (C=O), 1598 (C=N), 842, 732; ^1H NMR (300 MHz, $CDCl_3$): δ 1.66 (t, 2H, CH_3), 2.24 (q, 2H, CH_2), 2.56 (t, J = 7.7, 2H, C–CH_2), 2.94 (t, J = 7.7, 2H, CH_2–CO), 7.20–7.94 (m, 9H, Ar–H), 10.82 (s, 1H, CSNH); Mass (m/z): 377/378 (M^+/M^++1); Anal. Calcd for $C_{20}H_{19}N_5OS$: C, 63.64; H, 5.07; N, 18.55. Found: C, 63.58; H, 4.96; N, 18.42.
Compound ***4k****:* Yield: 48%; mp 202–204 °C; R_f 0.60; IR (KBr) υ_{max} (cm^{-1}): 3458 (NH), 3028 (CH), 2312 (C=S), 1612 (C=O), 1588 (C=N), 848, 758; ^1H NMR (300 MHz, $CDCl_3$): δ 1.16 (t, 3H, CH_3), 2.28 (q, 2H, CH_2), 2.58 (t, J = 7.8, 2H, C–CH_2), 3.02 (t, J = 7.8, 2H, CH_2–CO), 7.14–7.92 (m, 8H, Ar–H), 10.72 (s, 1H, CSNH); Mass (m/z): 411 (M^+); Anal. Calcd for $C_{20}H_{18}ClN_5OS$: C, 58.32; H, 4.40; N, 17.00. Found: C, 58.22; H, 4.32; N, 16.88.
Compound ***4l****:* Yield: 52%; mp 206–208 °C; R_f 0.64; IR (KBr) υ_{max} (cm^{-1}): 3456 (NH), 2936 (CH), 2344 (C=S), 1670 (C=O), 1608 (C=N); ^1H NMR (300 MHz, $CDCl_3$): δ 1.14 (t, 3H, CH_3), 2.24 (q, 2H, CH_2), 2.40 (s, 3H, CH_3), 2.56 (t, J = 7.8, 2H, C–CH_2), 2.98 (t, J = 7.8, 2H, CH_2–CO), 7.20–7.94 (m, 8H, Ar–H), 10.80 (s, 1H, CSNH); Mass (m/z): 391/392 (M^+/M^++1); Anal. Calcd for $C_{21}H_{21}N_5OS$: C, 64.43; H, 5.41; N, 17.89. Found: C, 64.36; H, 5.22; N, 17.82.
Compound ***4m****:* Yield: 52%; mp 206–208 °C; R_f 0.64; IR (KBr) υ_{max} (cm^{-1}): 3424 (NH), 3092 (CH), 2362 (C=S), 1654 (C=O), 1610 (C=N), 882; ^1H NMR (300 MHz, $CDCl_3$): δ 0.89 (d, 6H, 2 × CH_3), 1.64–1.72 (m, 1H, CH), 1.86 (t, 2H, CH_2), 2.96 (t, J = 7.6, 2H, C–CH_2), 3.20 (t, J = 7.6, 2H, CH_2–CO), 7.20–7.94 (m, 9H, Ar–H), 10.82 (s, 1H, CSNH); Mass (m/z): 405/406 (M^+/M^++1); Anal. Calcd for $C_{22}H_{23}N_5OS$: C, 65.15; H, 5.72; N, 17.27. Found: C, 65.08; H, 5.58; N, 17.16.
Compound ***4n****:* Yield: 48%; mp 210–212 °C; R_f 0.56; IR (KBr) υ_{max} (cm^{-1}): 3490 (NH), 2922 (CH), 2362 (C=S), 1632 (C=O), 1560 (C=N), 806; ^1H NMR (300 MHz, $CDCl_3$): δ 0.88 (d, 6H, 2 × CH_3), 1.68–1.74 (m, 1H, CH), 1.88 (t, 2H, CH_2), 2.86 (t, J = 7.8, 2H, C–CH_2), 3.24 (t, J = 7.8, 2H, CH_2–CO), 7.18–7.84 (m, 8H, Ar–H), 10.84 (s, 1H, CSNH); Mass (m/z): 439 (M^+); Anal. Calcd for $C_{22}H_{22}ClN_5OS$: C, 60.06; H, 5.04; N, 15.92. Found: C, 59.96; H, 4.92; N, 15.86.
Compound ***4o****:* Yield: 56%; mp 218–220 °C; R_f 0.58; IR (KBr) υ_{max} (cm^{-1}): 3442 (NH), 2926 (CH), 2356 (C=S), 1676 (C=O), 1616 (C=N); ^1H NMR (300 MHz, $CDCl_3$): δ 0.89 (d, 6H, 2 × CH_3), 1.66–1.74 (m, 1H, CH), 1.86 (t, 2H, CH_2), 2.40 (s, 3H, CH_3), 2.86 (t, J = 7.8, 2H, C–CH_2), 3.22 (t, J = 7.8, 2H, CH_2–CO), 7.20–7.94 (m, 8H, Ar–H), 10.86 (s, 1H, CSNH); Mass (m/z): 419/420 (M^+/M^++1); Anal. Calcd for $C_{23}H_{25}N_5OS$: C, 65.84; H, 6.01; N, 16.69. Found: C, 65.78; H, 5.82; N, 16.54.
Compound ***4p****:* Yield: 56%; mp 174–176 °C; R_f 0.60; IR (KBr) υ_{max} (cm^{-1}): 3210 (NH), 3096 (CH), 2371 (C=S), 1670 (C=O), 1496 (C=N); ^1H NMR (300 MHz, $CDCl_3$): δ 2.50 (t, J = 7.7, 2H, C–CH_2), 2.96 (t, J = 7.7, 2H, CH_2–CO), 7.12–7.94 (m, 14H, Ar–H), 10.82 (s, 1H, CSNH); Mass (m/z): 425/426 (M^+/M^++1); Anal. Calcd for $C_{24}H_{19}N_5OS$: C, 67.74; H, 4.50; N, 16.46. Found: C, 67.68; H, 4.38; N, 16.26.
Compound ***4q****:* Yield: 42%; mp 193–195 °C; R_f 0.58; IR (KBr) υ_{max} (cm^{-1}): 3426 (NH), 3094 (CH), 2366 (C=S), 1664 (C=O), 1592 (C=N), 844; ^1H NMR (300 MHz, $CDCl_3$): δ 2.46 (t, J = 7.6, 2H, C–CH_2), 2.98 (t, J = 7.6, 2H, CH_2–CO), 7.04–7.96 (m, 13H, Ar–H), 10.74 (s, 1H, CSNH); Mass (m/z): 459 (M^+); Anal. Calcd for $C_{24}H_{18}ClN_5OS$: C, 62.67; H, 3.94; N, 15.23. Found: C, 62.54; H, 3.88; N, 45.14.
Compound ***4r****:* Yield: 59%; mp 185–187 °C; R_f 0.62; IR (KBr) υ_{max} (cm^{-1}): 3446 (NH), 2928 (CH), 2374 (C=S), 1678 (C=O), 1610 (C=N); ^1H NMR (300 MHz, $CDCl_3$): δ 2.40 (s, 3H, CH_3), 2.52 (t, J = 7.7, 2H, C–CH_2), 2.96 (t, J = 7.7, 2H, CH_2–CO), 7.12–7.94 (m, 13H, Ar–H), 10.76 (s, 1H, CSNH); Mass (m/z): 439/440 (M^+/M^++1); Anal. Calcd for $C_{25}H_{21}N_5OS$: C, 68.32; H, 4.82; N, 15.93. Found: C, 68.14; H, 4.76; N, 15.86.
Compound ***4s****:* Yield: 38%; mp 194–196 °C; R_f 0.64; IR (KBr) υ_{max} (cm^{-1}): 3452 (NH), 2934 (CH), 1680 (C=O), 1612 (C=N), 1093, 846; ^1H NMR (300 MHz, $CDCl_3$): δ 2.46 (t, J = 7.8, 2H, C–CH_2), 2.90 (t, J = 7.8, 2H, CH_2–CO), 7.12–7.88 (m, 9H, Ar–H), 10.82 (s, 1H, CSNH); Mass (m/z): 383 (M^+); Anal. Calcd for $C_{18}H_{14}ClN_5OS$: C, 56.32; H, 3.68; N, 18.24. Found: C, 56.22; H, 3.54; N, 18.16.
Compound ***4t****:* Yield: 32%; mp 204–206 °C; R_f 0.62; IR (KBr) υ_{max} (cm^{-1}): 3454 (NH), 2924 (CH), 2358 (C=S), 1670 (C=O), 1612 (C=N), 1074, 836; ^1H NMR (300 MHz, $CDCl_3$): δ 2.54 (t, J = 7.8, 2H, C–CH_2), 2.98 (t, J = 7.8, 2H, CH_2–CO), 7.10–7.92 (m, 8H, Ar–H), 10.76 (s, 1H, CSNH); Mass (m/z): 418 (M^+); Anal. Calcd for $C_{18}H_{13}ClN_5OS$: C, 51.68; H, 3.13; N, 16.74. Found: C, 51.56; H, 3.02; N, 16.58.
Compound ***4u****:* Yield: 47%; mp 206–208 °C; R_f 0.66; IR (KBr) υ_{max} (cm^{-1}): 3450 (NH), 2938 (CH), 2356 (C=S), 1682 (C=O), 1614 (C=N); ^1H NMR (300 MHz, $CDCl_3$): δ 2.40 (s, 3H, CH_3), 2.56 (t, J = 7.7, 2H, C–CH_2), 2.98 (t, J = 7.7, 2H, CH_2–CO), 7.12–7.88 (m, 8H, Ar–H), 10.84 (s, 1H, CSNH); Mass (m/z): 397/399 (M^+/M^++2); Anal. Calcd for $C_{19}H_{16}ClN_5OS$: C, 57.35; H, 4.05; N, 17.60. Found: C, 57.26; H, 3.95; N, 17.47.
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