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ORIGINAL RESEARCH

Synthesis and anthelmintic activity of some novel (*E*)-2-methyl/propyl-4-(2-(substitutedbenzylidene)hydrazinyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidines

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Abstract

A series of some novel (*E*)-2-methyl/propyl-4-[(2-(substitutedbenzylidene)hydrazinyl]-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidines was synthesized and characterized by adopting an appropriate synthetic scheme. The effect of electron withdrawing and electron donating groups at the aromatic ring in presence of methyl and propyl substituents at the 2-position of the scaffold was evaluated for anthelmintic activity against adult Indian earthworms (*Pheretima posthuma*). Among 2-methyl-thieno[2,3-*d*]pyrimidine analogs, compounds with electron donating methoxy group either at *para*-position or at *meta* and *para*-positions of the aromatic ring showed potent anthelmintic activity at 100 µg/ml (284 and 261.8 µM concentrations) for compounds **5g** and **5h** with a mean paralytic time of 2.32, 2.22 min, and helminthicidal time of 22.38, 19.43 min, respectively. In contrast, the presence of electron withdrawing chloro group at *ortho* and *para*-position of the aromatic ring was found to be favorable for the anthelminthic activity of the compounds **5n** and **5o** (at concentration of 259.7 µM) with propyl group at the 2-position of the thieno[2,3-*d*]pyrimidine scaffold, exhibiting mean paralytic time of 2.5 min, 2.81 min and helminthicidal time of 21, 20.03 min, respectively. Anthelmintic activities of these four compounds **5g**, **5h**, **5n**, and **5o** (at the concentrations of 284, 261.8, 259.7, and 259.7 µM, respectively) were found to be on par with the standard drug piperazine adipate (time for paralysis and death at 6.25 and 24.5 min, respectively) at concentration of 100 µg/ml (431.03 µM). Overall, the potency of these compounds (**5g**, **5h**, **5n**, and **5o**) is better than standard drug as they exhibited the same activity at 259.7–284 µM as that of a standard drug (which has shown the same activity at 431.03 µM). Further, the predicted ADME properties of all the synthesized compounds were found to be in the satisfactory ranges as predicted by SwissADME software and found to have drug-like properties. Thus, further modification of these compounds might lead to the discovery of more potent analogs.

Keywords Anthelmintics · Thienopyrimidines · Benzylidenehydrazines · ADME properties by SwissADME software

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Introduction

Helminthiasis is one of the most common parasitic infections and a neglected tropical disease, mainly affecting the poor people and livestock of developing countries due to inadequate sanitation and unhygienic conditions (Knox et al. 2012; Woods et al. 2007; Le et al. 2019; Idris et al. 2019). The helminth parasites mainly reside in the gastrointestinal tract (GIT) of the host, but their larvae can also invade the liver and other organs of the body (Tripathi 2013; Stear et al. 2007). They are harmful to the host by causing malnutrition, anemia, secreting toxins and if untreated, then they can also result in chronic infections by regulating immune-mediated inflammation (Elliott and Weinstock 2012; Jones and Berkley 2014; Helmby 2015). Furthermore, the fecal elimination of helminth eggs from the infected people and animals can contaminate the soils in areas with poor sanitation conditions leading to the reinfection in environment (Chander et al. 2014; Idris et al. 2019). The World Health Organization (WHO) estimates that more than 1.5 billion people, or 24% of the world's population harbor soil-transmitted worm infections worldwide, including more than 267 million preschool-age children and over 568 million school-age children as well as women of child bearing age, pregnant women including and occupational groups like fishermen and irrigation field workers for schistosomiasis https://www.who.int/neglected_diseases/news/nobel_prize_2019/en/ (accessed on 15/1/2019).

Anthelmintic drugs can either kill (vermicides) or expel the worms from the GIT and other parts of the body. There are four major classes of common anthelmintics viz. benzimidazoles (e.g., mebendazole, albendazole, fenbendazole, and flubendazole), imidazothiazoles (levamisole), piperazines (phosphate, citrate, and adipate), piperidines (pyrantel and oxantel), macrocyclic lactones (ivermectin and moxidectin), and the amino-acetonitrile derivative, monepantel available for the treatment of nematodes infections (Taman and Azab 2014; Geary et al. 2015). Surprisingly, many of these marketed drugs available for human treatment were first developed as veterinary medicines (Zajíčková et al. 2020). However, most of these drugs are associated with undesirable side effects in the GIT (nausea, vomiting, and abdominal pain), liver, and kidney, including allergic reactions, alopecia, hypotension, and body ache among others. In addition, helminths have been gaining resistance against many drugs including the latest drug monepentel and moxidectin, thereby imposing a big challenge to the chemists for the discovery and development of alternative drugs with improved efficacy, pharmacokinetic and pharmacodynamics properties, devoid of side effects of existing anthelmintic drugs (Kotze and Prichard 2016; Geary 2012; Shalaby 2013; Raza et al. 2016; Lanusse et al. 2018; Martin et al. 2018). It should be noted that anthelmintic drugs mainly acting on the GIT are structurally different showing different mechanism of actions. In recent years, many plants, herbal extracts, and novel chemical compounds belonging to diverse heterocyclic scaffolds have been explored for their antiparasitic and anthelmintic activities (Zajíčková et al. 2020; Romero-Benavides et al. 2017; ba Ndob et al. 2016; Santos et al. 2019; Bauri et al. 2015; Keiser et al. 2016; Preston et al. 2017; Preston and Gasser 2018; Taylor et al. 2013; Tyagi et al. 2018). Interestingly, some of the recently reported molecules with thieno[2,3-*d*]pyrimidine scaffold have shown promising anthelmintic activities (Meyer et al. 2009; Mavrova et al. 2018; Bahashwan et al. 2013; Partridge et al. 2018). These encouraging results gave us an impetus to synthesize and evaluate the anthelmintic activities of a novel series of (*E*)-2-methyl/propyl-4-(2-(substitutedbenzylidene)hydrazinyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidines.

Materials and methods

Chemistry

Experimental

Reactions were routinely monitored by thin layer chromatography (TLC) on silica gel (precoated F254 Merck plates) and the spots were visualized under UV light (254 nm). Melting points were recorded in open capillaries on LABINDIA melting point apparatus and were uncorrected. IR spectra were recorded on Bruker FT-IR Spectrometer (Spectrum RX I) using the KBr pellet technique. ^{1}H NMR spectra were determined in $CDCl_3$ or DMSO-d_6 with a Bruker Avance II [400 MHz (^{1}H)] and signals were recorded in parts per million (δ) downfield from tetramethylsilane (TMS) as an internal standard. Mass spectra (ESI) were recorded on Waters Micromass Q-TOF Micro.

General procedure for the synthesis of compounds

2-Amino-3-carbethoxy-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**1**) (Gewald et al. 1966; Gewald 1965; Rao et al. 2013), 4-hydroxy-2-methyl/propyl-5,6,7,8-tetrahydrobenzo[4,5]thieno [2,3-*d*]pyrimidines (**2a** and **2b**) (Prasad et al. 2001; Prasad and Kishore 2007a), 4-chloro-2-methyl/propyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidines (**3a** and **3b**) (Deb et al. 2018; Prasad et al. 2001), 4-hydrazino-2-methyl/propyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidines (**4a** and **4b**) (Prasad et al. 2008, 2007b) were prepared by following reported procedures.

General procedure for the synthesis of 2-methyl/propyl-4-(2-(substitutedbenzylidene)hydrazinyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*] pyrimidines (5a–p)

Compounds **4a** and **4b** (0.002 M, 0.5 g) and appropriate aldehyde (0.002 M) were taken in a round bottom flask containing 20 ml ethanol and 2 drops of glacial acetic acid was added and kept in stirring for about 2 h. The reaction was monitored using TLC. After completion of the reaction the reaction mixture was kept at room temperature overnight. The precipitate formed was filtered, dried and recrystallized with acetone to get the final products **5a–p**.

(*E*)-4-(2-benzylidenehydrazinyl)-2-methyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*] pyrimidine(5a) White solid, yield: 73%, M.P.: 122–123 °C, IR (KBr) ν_{max}: 3433.94, 2931.10, 2833.56, 1628.42, 1544.92 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) $\delta = 11.13$ (1H, s, HN–N=CH–), 8.41 (1H, s, HN–N=CH),7.98–7.99 (2H, d, $J = 6.4$ Hz, 2ArH), 7.41–7.46 (3H, q, $J = 7.4$ Hz, 2ArH), 2.98–3.00 (2H, m, CH_2), 2.73–2.75 (2H, m, CH_2), 2.42 (3H, s, CH_3), 1.79–1.82 (4H, m, $2CH_2$) ppm; MS (ESI) *m/z* (%): 322 (M^+, 100); 323 (M + H, 30); Elemental analysis: calcd. For $C_{18}H_{18}N_4S$: C, 67.08; H, 5.59; N, 17.39%. Found: C, 67.10; H, 5.61; N, 17.41%.

(*E*)-4-((2-(2-methyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-yl)hydrazono)methyl)phenol (5b) White solid, yield: 70%, M.P.: 215–216 °C, IR (KBr) ν_{max}: 3379.02, 2935.74, 1565.62, 1415.74 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) $\delta = 10.62$ (s, 1H, OH), 8.57 (1H, s, HN–N=CH–), 8.19 (1H, s, HN–N=CH–), 7.55–7.57 (2H, d, $J = 8.2$ Hz, 2ArH), 6.73–6.75 (2H, d, $J = 8.4$ Hz, 2ArH), 2.67 (3H, s, –CH_3), 2.62–2.67 (2H, m, CH_2), 2.25–2.2.58 (2H, m, CH_2), 1.70–1.75 (4H, m, $2CH_2$) ppm; MS (ESI) *m/z* (%)**:** 338 (M^+, 100); 339 (M + H, 40); Elemental analysis: calcd. For $C_{18}H_{18}N_4OS$: C, 63.90; H, 5.33; N, 16.57%. Found: C, 63.92; H, 5.35; N, 16.60%.

(*E*)-2-methyl-4-(2-(4-nitrobenzylidene)hydrazinyl)-5,6,7,8-tetrahydrobenzo[4,5] thieno[2,3-*d*]pyrimidine (5c) Light yellow solid, yield: 76%, M.P.: 210–211 °C, IR (KBr) ν_{max}: 3319, 2931.96, 1622.62, 1556.97 cm^{-1}; ^1H NMR (Acetone-d_6, 400 MHz) $\delta = 10.95$ (1H, s, HN–N=CH–), 8.38 (1H, s, HN–N=CH), 8.11–8.14 (2H, d, $J = 8.8$, 2ArH), 7.95–7.97 (2H, d, $J = 8.4$, 2ArH), 2.92–2.94 (2H, m, CH_2), 2.66–2.68 (2H, m, CH_2), 2.31 (3H, s, –CH_3), 1.72–1.77 (4H, m, $2CH_2$) ppm; MS (ESI) *m/z* (%)**:** 367 (M^+ 100); 368 (M + H, 20); Elemental analysis: calcd. For $C_{18}H_{17}N_5O_2S$: C, 58.86; H, 4.63; N, 19.07%. Found: C, 58.89; H, 4.66; N, 19.17%.

(*E*)-2-methyl-4-(2-(3-nitrobenzylidene)hydrazinyl)-5,6,7,8-tetrahydrobenzo[4,5] thieno[2,3-*d*]pyrimidine (5d) Light yellow solid, yield: 75%, M.P.: 198–199 °C, IR (KBr) ν_{max}: 3350.07, 2932.93, 1629.25, 1521.53 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) $\delta = 11.37$ (1H, s, HN–N=CH–), 8.78 (1H, s, HN–N=CH), 8.55 (1H, s, ArH), 8.44–8.46 (1H, d, $J = 8.2$ Hz, ArH), 8.22–8.24 (1H, d, $J = 8.2$ Hz, ArH), 7.71–7.76 (1H, t, $J = 8.2$, ArH), 2.99–3.01 (2H, m, CH_2), 2.74–2.76 (2H, m, CH_2), 2.45 (3H, s, –CH_3), 1.78–1.79 (4H, d, $J = 4.8$, $2CH_2$) ppm; MS (ESI) *m/z* (%): 367 (M^+, 100); 368 (M + H, 20); Elemental analysis: calcd. For $C_{18}H_{17}N_5O_2S$: C, 58.86; H, 4.63; N, 19.07%. Found:C, 58.87; H, 4.65; N, 19.20%.

(*E*)-4-(2-(4-chlorobenzylidene)hydrazinyl)-2-methyl-5,6,7,8-tetrahydrobenzo[4,5] thieno[2,3-*d*]pyrimidine (5e) Light yellow solid, yield: 81%, M.P.: 170–171 °C, IR (KBr) ν_{max}: 3335.58, 2923.26, 1625.61, 726.79 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) $\delta = 11.19$ (1H, s, HN–N=CH–), 8.41 (1H, s, HN–N=CH), 8.01–8.03 (2H, d, $J = 8.0$ Hz, 2ArH), 7.49–7.51 (2H, d, $J = 8.4$ Hz, 2ArH), 2.97–2.99 (2H, m, CH_2), 2.73–2.75 (2H, m, CH_2), 2.42 (3H, s, –CH_3), 1.78–179 (4H, m, $2CH_2$) ppm; MS (ESI) *m/z* (%)**:** 357 (M^+, 100); Elemental analysis: calcd. For $C_{18}H_{17}ClN_4S$: C, 60.50; H, 4.76; N, 15.69%. Found: C, 60.54; H, 4.80; N, 15.72%.

(*E*)-4-(2-(2-chlorobenzylidene)hydrazinyl)-2-methyl-5,6,7,8-tetrahydrobenzo[4,5] thieno[2,3-*d*]pyrimidine (5f) Light brown solid, yield: 77%, M.P.: 154–156 °C, IR (KBr) ν_{max}: 3323.23, 2920.41, 1625.57, 752.91 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) $\delta = 11.27$ (1H, s, HN–N=CH–), 8.67 (1H, s, HN–N=CH–) 8.53–8.51 (1H, m, ArH), 7.52–7.50 (1H, m, ArH), 7.44–7.42 (2H, m, ArH), 2.98–2.99 (2H, m, CH_2), 2.73–2.74 (2H, m, CH_2), 2.43 (3H, s, –CH_3), 1.79–1.81 (4H, m, $2CH_2$) ppm; MS (ESI) *m/z* (%): 356 (M^+, 100); 357 (M + H, 30); Elemental analysis: calcd. For $C_{18}H_{17}ClN_4S$: C, 60.50; H, 4.76; N, 15.69%. Found: C, 60.48; H, 4.80; N, 15.70%.

(*E*)-4-(2-(4-methoxybenzylidene)hydrazinyl)-2-methyl-5,6,7,8-tetrahydrobenzo[4,5] thieno[2,3-*d*]pyrimidine (5g) Light brown solid, yield: 72%, M.P.: 98–99 °C, IR (KBr) ν_{max}: 3393.00, 2934.19, 2835.85, 1609.44 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) $\delta = 11.05$ (1H, s, HN–N=CH–), 8.35 (1H, s, HN–N=CH–) 7.91–7.93 (2H, d, $J = 8.0$ Hz, 2ArH), 6.99–7.01 (2H, d, $J = 8.4$ Hz, 2ArH), 3.81 (3H, s, –OCH_3), 2.97–2.99 (2H, m, CH_2), 2.72–2.74 (2H, m, CH_2), 2.40 (3H, s, –CH_3), 1.78–1.79 (4H, m, $2CH_2$) ppm; MS (ESI) *m/z* (%): 352 (M^+, 100); 353 (M + H, 30); Elemental analysis: calcd. For $C_{19}H_{20}N_4OS$: C, 64.78; H, 5.68; N, 15.91%. Found: C, 64.82; H, 5.72; N, 15.95%.

(*E*)-4-(2-(3,4-dimethoxybenzylidene)hydrazinyl)-2-methyl-5,6,7,8-tetrahydrobenzo [4,5]thieno[2,3-*d*]pyrimidine (5h)

Light brown solid, yield: 73%, M.P.: 120–121 °C, IR (KBr) ν_{max}: 3359.82, 2938.52, 2825, 1601.50 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) $\delta = 11.10$ (1H, s, HN–N=CH–), 8.34 (1H, s, HN–N=CH–) 7.68–7.69 (1H, d, $J = 8.6$ Hz, ArH), 7.38–7.39 (1H, s, ArH), 7.00–7.02 (1H, d, $J =$ 8.4 Hz, ArH), 3.86 (3H, s, $–OCH_3$), 3.81 (3H, s, $–OCH_3$), 3.86 (3H, s, $–OCH_3$), 2.97–2.98 (2H, m, $–CH_2$), 2.72–2.74 (2H, m, $–CH_2$), 2.40 (3H, s, $–CH_3$), 1.78–1.80 (4H, m, $2CH_2$) ppm; MS (ESI) *m/z* (%): 382 (M^+, 100); 383 (M + H, 30); Elemental analysis: calcd. For $C_{20}H_{22}N_4O_2S$: C, 62.83; H, 5.76; N, 14.66%. Found: C, 62.87; H, 5.78; N, 14.70%.

(*E*)-4-(2-benzylidenehydrazinyl)-2-propyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*] pyrimidine (5i) Light brown solid, yield: 72%, M.P.: 102–103 °C, IR (KBr) ν_{max}: 3348.85, 1628.86, 1583.73, 1545.31, 2926.67, 2956.35 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) $\delta = 11.02$ (1H, s, HN–N=CH–), 8.41 (1H, s, HN–N=CH–), 7.41–7.46 (1H, m, ArH), 7.67–7.70 (2H, d, $J = 7.6$ Hz, ArH), 7.97–7.98 (2H, d, $J = 7.2$ Hz, 2ArH), 2.98–3.02 (2H, m, $–CH_2CH_2CH_3$), 2.77–2.79 (2H, m, $–CH_2CH_2CH_3$), 1.69–1.78 (8H, m, $4CH_2$), 0.94–0.98 (3H, m, $–CH_2CH_2CH_3$) ppm; MS (ESI) *m/z* (%): 350 (M^+, 100); Elemental analysis: calcd. For $C_{20}H_{22}N_4S$: C, 68.57; H, 6.29; N, 16.00%. Found: C, 68.60; H, 6.32; N, 16.10%.

(*E*)-4-((2-(2-propyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-yl) hydrazono)methyl)phenol (5j) Light brown solid, yield: 76%, M.P.: 210–212 °C, IR (KBr) ν_{max}: 3370.58, 1414.54, 1603.84, 2946.17 cm^{-1}; ^1H NMR (Acetone-d_6, 400 MHz) $\delta = 10.53$ (1H, s, OH), 8.53 (1H, s, HN–N=CH–), 8.19 (1H, s, HN–N=CH–),7.54–7.56 (2H, d, $J = 8.0$ Hz, 2ArH), 6.74–6.76 (2H, d, $J = 8.4$ Hz, 2ArH), 2.90–2.92 (2H, m, CH_2), 2.68–2.70 (2H, m, $CH_2CH_2CH_3$), 2.63–2.65 (2H, m, $CH_2CH_2CH_3$), 1.63–1.70 (6H, m, $3CH_2$), 0.85–0.88 (3H, t, $J = 7.2$ Hz, $CH_2CH_2CH_3$) ppm; MS (ESI) *m/z* (%): 366 (M^+, 100); Elemental analysis: calcd. For $C_{20}H_{22}N_4OS$: C, 65.57; H, 6.01; N, 15.30%. Found: C, 65.61; H, 6.21; N, 15.35%.

(*E*)-4-(2-(4-nitrobenzylidene)hydrazinyl)-2-propyl-5,6,7,8-tetrahydrobenzo [4,5]thieno[2,3-*d*]pyrimidine (5k) Light brown solid, yield: 80%, M.P.: 180–182 °C, IR (KBr) ν_{max}: 3343.75, 1623.1, 2918.06, 2931.27, 1530.45 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) $\delta = 10.83$ (1H, s, HN–N=CH–), 8.38 (1H, s, HN–N=CH–), 8.11–8.13 (2H, d, $J = 8.8$ Hz, 2ArH), 7.94–7.96 (2H, d, $J = 8.4$ Hz, 2ArH), 2.92 (2H, m, CH_2), 2.66–2.67 (2H, m, $-CH_2CH_2CH_3$), 2.57–2.61 (2Hm, $–CH_2CH_2CH_3$), 1.63–1.70 (6H, m, $3CH_2$), 0.85–0.88 (3H, t, $J = 7.4$, $–CH_2CH_2CH_3$) ppm; MS (ESI) *m/z* (%): 395 (M^+, 100); Elemental analysis: calcd. For $C_{20}H_{21}N_5O_2S$: C, 60.76, H, 5.32; N, 17.72%. Found: C, 60.72, H, 5.30; N, 17.70%.

(*E*)-4-(2-(3-nitrobenzylidene)hydrazinyl)-2-propyl-5,6,7,8-tetrahydrobenzo [4,5]thieno[2,3-*d*]pyrimidine (5l) Light brown solid, yield: 87%, M.P.: 160–161 °C, IR (KBr) ν_{max}: 3338.05, 1629.44, 2935.38, 1539.96 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) $\delta = 11.24$ (1H, s, HN–N=CH–), 8.76 (1H, s, HN–N=CH–), 8.55 (1H, s, ArH), 8.44–8.46 (1H, d, $J = 8.0$ Hz, ArH), 8.21–8.24 (1H, m, ArH), 8.09–8.11 (1H, d, $J = 7.6$ Hz, ArH), 2.73–2.77 (4H, m, $2CH_2$), 1.78–1.82 (4H, m, $CH_2CH_2CH_3$), 1.70–1.74 (3H t, *m*, $CH_2CH_2CH_3$), 0.92–0.98 (4H, t, *m*, $2CH_2$) ppm; MS (ESI) *m/z* (%): 395 (M+, 100); Elemental analysis: calcd. For $C_{20}H_{21}N_5O_2S$: C, 60.76, H, 5.32; N, 17.72%. Found: C, 60.80, H, 5.35; N, 17.76%.

(*E*)-4-(2-(2-nitrobenzylidene)hydrazinyl)-2-propyl-5,6,7,8-tetrahydrobenzo [4,5]thieno[2,3-*d*]pyrimidine (5m) Light brown solid, yield: 83%, M.P.: 134–136 °C, IR (KBr) ν_{max}: 3324.05, 1629.84, 2926.21, 1528.50 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) $\delta = 11.12$ (1H, s, HN–N=CH–), 8.64 (1H, s, HN–N=CH–), 8.53–8.55 (1H, m, ArH), 8.01–8.04 (1H, m, ArH), 7.80–7.82 (1H, m, ArH), 7.64 (1H m, ArH), 2.71–2.76 (4H, q, $J = 6.6$ Hz, $2CH_2$), 1.76–1.82 (4H, q, $J = 8.1$ Hz, $–CH_2CH_2CH_3$), 1.70–1.74 (3H, t, $J =$ 7.6 Hz, $–CH_2CH_2CH_3$), 0.92–0.98 (4H m, $2CH_2$) ppm; MS (ESI) *m/z* (%): 395 (M+, 100); Elemental analysis: calcd. For $C_{20}H_{21}N_5O_2S$: C, 60.76, H, 5.32; N, 17.72%. Found: C, 60.82, H, 5.36; N, 17.78%.

(*E*)-4-(2-(4-chlorobenzylidene)hydrazinyl)-2-propyl-5,6,7,8-tetrahydrobenzo [4,5]thieno[2,3-*d*]pyrimidine(5n) Light brown solid, yield: 83%, M.P.: 132–134 °C, IR (KBr) ν_{max}: 3268, 1625.84, 2934.2, 2950.27, 743.83 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) $\delta = 11.07$ (1H, s, HN–N=CH–), 8.41 (1H, s, HN–N=CH–), 8.00–8.02 (2H, d, $J = 8.4$ Hz, 2ArH), 7.50–7.52 (2H, d, $J = 8.2$ Hz, 2ArH), 2.79–2.80 (2H, m, $CH_2CH_2CH_3$), 2.70–2.77 (2H, m, $CH_2CH_2CH_3$), 1.69–1.78 (8H, m, $4CH_2$), 0.94–0.98 (3H, m, $CH_2CH_2CH_3$) ppm; MS (ESI) *m/z* (%): 385 (M+, 100); Elemental analysis: calcd. For $C_{20}H_{21}ClN_4S$: C, 62.34; H, 5.46, N, 14.55%. Found: C, 62.40; H, 5.48, N, 14.58%.

(*E*)-4-(2-(2-chlorobenzylidene)hydrazinyl)-2-propyl-5,6,7,8-tetrahydrobenzo [4,5]thieno[2,3-*d*]pyrimidine(5o) Light brown solid, yield: 75%, M.P.: 110–112 °C, IR (KBr) ν_{max}: 3354.05, 1624.7, 2930.78, 2955.28, 750.72 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) $\delta = 11.16$ (1H, s, HN–N=CH–), 8.67 (1H, s, HN–N=CH–), 8.48–8.51 (1H t, $J = 4.6$ Hz, ArH), 7.50–7.54 (1H, m, ArH), 7.42–7.44 (2H, t, $J =$ 4.6 Hz, 2ArH), 2.71–2.75 (4H, t, $J = 7.4$ Hz, $2CH_2$),

1.75–1.82 (4H, m, $CH_2CH_2CH_3$), 1.69–1.73 (3H, t, $J =$ 7.4 Hz, $CH_2CH_2CH_3$), 0.92–0.97 (4H, m, $2CH_2$) ppm; MS (ESI) *m/z* (%): 385 (M+, 100); Elemental analysis: calcd. For $C_{20}H_{21}ClN_4S$: C, 62.34; H, 5.46, N, 14.55%. Found: C, 62.38; H, 5.50, N, 14.60%.

(*E*)-4-(2-(3,4-dimethoxybenzylidene)hydrazinyl)-2-propyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidine (5p) Light brown solid, yield: 86%, M.P.: 130–132 °C, IR (KBr) ν_{max}:3346.95, 1627.2, 2926.32, 2946.97, 2846.74 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) $\delta = 10.99$ (1H, s, HN–N=CH–), 8.34 (1H, s, HN–N=CH-), 8.24 (1H, s, ArH), 7.67–7.68 (1H, d, $J = 7.6$ Hz, ArH), 7.01–7.03 (1H, d, $J = 8.0$ Hz, ArH), 3.86 (3H, s, OCH_3), 3.81 (3H, s, $–OCH_3$), 2.67–2.75 (4H, m, CH_2), 1.75–1.83 (4H, m, $–CH_2CH_2CH_3$), 1.69–1.73 (3H, m, $–CH_2CH_2CH_3$), 0.92–0.97 (4H, m, $2CH_2$) ppm; MS (ESI) *m/z* (%): 410 (M^+100); 411 (M+H, 30); Elemental analysis: calcd. For $C_{22}H_{26}N_5O_2S$: C, 64.39; H, 6.34; N, 17.07%. Found: C, 64.41; H, 6.37; N, 17.10%.

Anthelmintic assay

All the target compounds (**5a–p**) were screened for anthelmintic activity as per the reported method (Ajaiyeoba et al. 2008; Garg et al. 2014) by using Indian earthworms (*Pheretima posthuma*), because of their anatomical and physiological similarity with human intestinal roundworm. All adult Indian earthworms of ~5–8 cm long and 0.4–0.8 mm diameter were collected from local Ramlakshmi vermi compost yard, Palakol. Earthworms were washed with normal saline water and kept in different groups of Petri dishes labeled as control, standard and tests, containing six worms in each plate. Piperazin adipate (37.0% w/w) was used as a standard drug at a concentration of 100 µg/ml (431.03 µM). The dose of piperazine adipate was optimized by carrying out its activity using different concentrations (10, 20, 50, 80, and 100 µg/ml). At 100 µg/ml, it showed activity and thus selected as standard dose. Dimethyl sulphoxide (DMSO) 1% was used as control. Test solutions of all the target compounds (**5a–p**) prepared with 1% DMSO were evaluated at four different concentrations of 20 µg/ml (48.7–62.1 µM), 50 µg/ml (122.0–155.2 µM), 80 µg/ml (195.1–248.4 µM), and 100 µg/ml (243.9–310.5 µM), respectively. Petri plates of the test groups were treated individually with 20 ml of test solutions of each concentration. Motility of worms was recorded after incubating them in a humid chamber with a thermostat at 37 °C (Mavrova et al. 2018). Time taken for complete paralysis and death of earthworms were recorded. Time taken for earthworms to become motionless (except vigorous shaking) was considered as the time of paralysis; whereas the time of death was ascertained based on the observation of complete lack of any movement of worms upon vigorous shaking followed by dipping in warm water (50 °C). *T*-test was run for the biological data of all the compounds. Results of anthelmintic activities are listed in Table 1.

Results and discussion

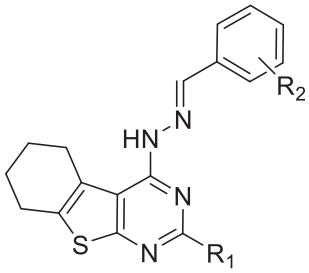
Chemistry

As illustrated in Fig. 1, the compound 2-amino-3-carbethoxy-4,5,6,7-tetrahydrobenzo[4,5]thiophene (**1**) was synthesized by stirring molar quantities of cyclohexanone, ethylcyanoacetate and sulfur in ethanol for one hour under basic conditions following the reported procedure (Gewald et al. 1966; Gewald 1965). Compounds (**2a**) and (**2b**) were prepared by condensing compound (**1**) with acetonitrile and butyronitrile, respectively, in the presence of dry hydrogen chloride. Chlorination of compounds **2a** and **2b** with phosphorous oxychloride at 110 °C for 6 h under anhydrous conditions resulted in the formation of 4-chloro-2-methyl/propyl-5,6,7,8-tetrahydrobenzo[*b*]thieno[2,3-*d*]pyrimidines (**3a** and **3b**), which upon further treatment with hydrazine hydrate resulted in the formation of key intermediate compounds 4-hydrazino-2-methyl/propyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidines (**4a** and **4b**). Compounds **2a**, **2b**, **3a**, **3b**, **4a**, and **4b** were also synthesized by following our previously reported procedure and their melting points were corroborated well with the melting point of these compounds (Prasad et al. 2001, 2008). Compounds **4a** and **4b** were treated with various aldehydes to obtain the target compounds **5a–p**, which were characterized based on their spectrometric analysis.

Anthelmintic activity

All the target compounds (**5a–p**) were screened for their preliminary anthelmintic activity (Table 1) following the reported method (Ajaiyeoba et al. 2008) by using adult Indian earthworms (*P. posthuma*) because of its anatomical and physiological similarity with the human intestinal roundworm parasites. Piperazine adipate was used as a standard at 100 µg/ml (431.03 µM) concentration. All the synthesized compounds were evaluated at four different concentrations of 20 (48.7–62.1 µM) 50 (122.0–155.2 µM), 80 (195.1–248.4 µM), and 100 (243.9–310.5 µM) µg/ml. Six worms were used for each concentration and the time taken for paralysis and death were recorded in minutes. The standard drug piperazine adipate (100 µg/ml, 431.03 µM) showed paralytic activity at a mean time of 6.25 min. At the same concentration [100 µg/ml (243.9–295.8 µM)], compounds **5b**, **5e**, **5f**, **5g**, **5h**, **5k**, **5l**, **5m**, **5n**, **5o**, **5p** paralyzed

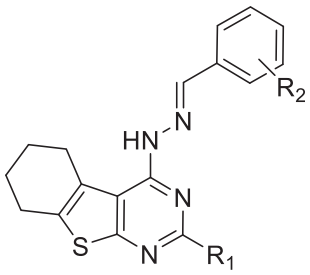
Table 1 Anthelmintic activity of (*E*)-2-methyl/propyl-4-(2-(substitutedbenzylidene)hydrazinyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*] pyrimidines 5a–5p



(**5a-p**)

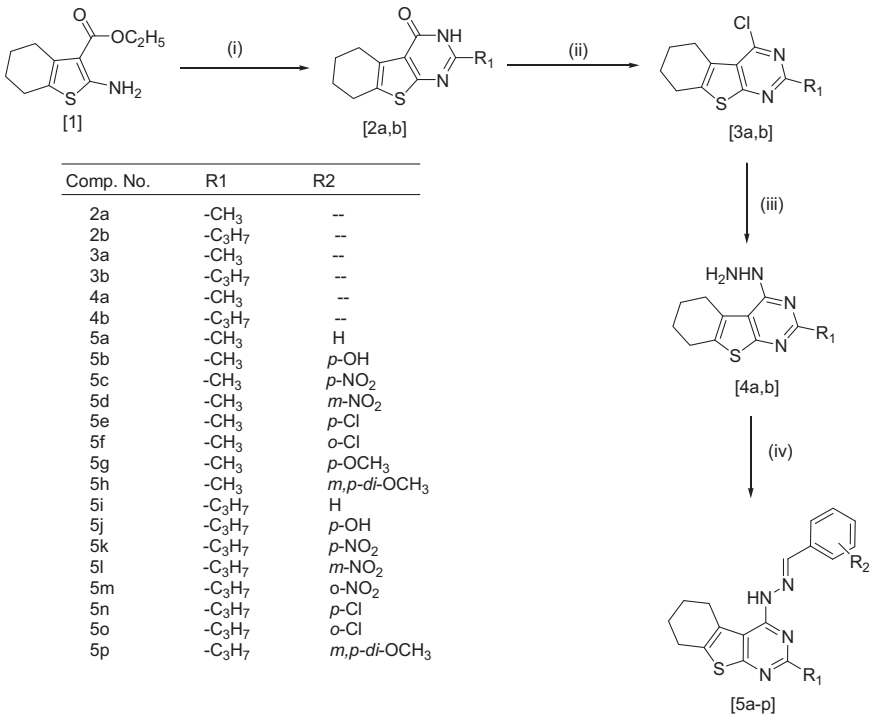
Comp.	R_1	R_2	Concentration (μg/ml)	Concentration (μM)	Time taken for paralysis in minutes (Mean and S.E.M.)	Time taken for death in minutes (Mean and S.E.M.)
5a	$-CH_3$	H	20	62.1	30.06 ± 0.006	110.35 ± 0.076
			50	155.2	33.37 ± 0.065	80.74 ± 0.205
			80	248.4	14.03 ± 0.114	49.91 ± 0.153
			100	310.5	06.36 ± 0.174	40.22 ± 0.217
5b	$-CH_3$	*p*-OH	20	59.2	–	–
			50	147.9	11.46 ± 0.170	40.60 ± 0.748
			80	236.6	09.96 ± 0.290	57.85 ± 1.298
			100	295.8	04.24 ± 0.045	38.19 ± 0.045
5c	$-CH_3$	*p*-NO_2	20	54.5	42.83 ± 1.740	112.16 ± 4.020
			50	136.2	36.77 ± 1.007	102.66 ± 2.216
			80	218.0	15.60 ± 0.887	49.19 ± 3.619
			100	272.4	08.92 ± 1.655	25.31 ± 0.811
5d	$-CH_3$	*m*-NO_2	20	54.5	40.46 ± 0.119	110.45 ± 0.076
			50	136.2	34.14 ± 0.051	100.31 ± 0.117
			80	218.0	14.35 ± 0.272	48.49 ± 0.026
			100	272.4	08.72 ± 0.141	24.98 ± 0.177
5e	$-CH_3$	*p*-Cl	20	56.0	40.39 ± 0.065	110.58 ± 0.153
			50	140.0	34.14 ± 0.051	100.19 ± 0.067
			80	224.0	07.20 ± 0.057	93.04 ± 0.579
			100	280.1	05.58 ± 0.153	35.71 ± 0.202
5f	$-CH_3$	*o*-Cl	20	56.0	21.44 ± 1.147	99.14 ± 1.360
			50	140.0	27.85 ± 2.310	100.50 ± 1.176
			80	224.0	06.38 ± 0.314	83.18 ± 8.226
			100	280.1	05.43 ± 0.569	30.55 ± 2.576
5g	$-CH_3$	*p*-OCH_3	20	56.8	–	–
			50	142.0	09.58 ± 0.153	38.60 ± 0.200
			80	227.3	05.35 ± 0.012	28.86 ± 0.190
			100	284.0	02.32 ± 0.007	22.38 ± 0.144
5h	$-CH_3$	*m,p*-di-OCH_3	20	52.3	–	–
			50	130.9	09.2 ± 0.608	34.57 ± 1.095
			80	209.4	05.18 ± 0.464	21.88 ± 0.28
			100	261.8	02.22 ± 0.236	19.43 ± 0.168
5i	$-C_3H_7$	H	20	57.1	33.81 ± 0.580	96.86 ± 0.435

Table 1 (continued)



(**5a-p**)

Comp.	R_1	R_2	Concentration (μg/ml)	Concentration (μM)	Time taken for paralysis in minutes (Mean and S.E.M.)	Time taken for death in minutes (Mean and S.E.M.)
			50	142.9	33.16 ± 0.468	72.16 ± 0.557
			80	228.6	09.81 ± 0.183	63.13 ± 0.416
			100	285.7	06.08 ± 0.179	49.55 ± 1.079
5j	$-C_3H_7$	*p*-OH	20	54.6	43.00 ± 2.708	75.15 ± 1.306
			50	136.6	10.45 ± 0.062	96.49 ± 1.891
			80	218.6	06.43 ± 0.042	81.89 ± 0.740
			100	273.2	08.00 ± 1.366	73.55 ± 3.060
5k	$-C_3H_7$	*p*-NO_2	20	50.6	28.79 ± 1.691	75.49 ± 9.086
			50	126.6	23.06 ± 3.424	62.26 ± 6.232
			80	202.5	13.37 ± 1.274	47.59 ± 7.507
			100	253.2	05.39 ± 0.337	39.90 ± 7.745
5l	$-C_3H_7$	*m*-NO_2	20	50.6	35.87 ± 0.212	86.08 ± 0.453
			50	126.6	22.08 ± 0.591	61.67 ± 1.021
			80	202.5	13.01 ± 0.189	52.88 ± 1.109
			100	253.2	05.49 ± 0.286	43.36 ± 1.184
5m	$-C_3H_7$	*o*-NO_2	20	50.6	33.26 ± 0.358	84.09 ± 0.741
			50	126.6	24.97 ± 0.346	56.60 ± 1.720
			80	202.5	14.10 ± 0.312	51.75 ± 0.487
			100	253.2	05.41 ± 0.046	43.66 ± 0.455
5n	$-C_3H_7$	*p*-Cl	20	51.9	36.00 ± 0.620	63.53 ± 0.305
			50	129.9	05.31 ± 0.065	43.25 ± 0.349
			80	207.8	03.98 ± 0.150	24.00 ± 0.428
			100	259.7	02.50 ± 0.058	21.00 ± 0.546
5o	$-C_3H_7$	*o*-Cl	20	51.9	36.45 ± 1.970	69.35 ± 5.519
			50	129.9	05.40 ± 0.236	44.76 ± 2.364
			80	207.8	03.81 ± 0.321	23.40 ± 2.346
			100	259.7	02.81 ± 0.273	20.03 ± 2.745
5p	$-C_3H_7$	*m,p*-di-OCH_3	20	48.7	33.08 ± 1.012	86.60 ± 0.711
			50	122.0	29.73 ± 0.615	63.25 ± 0.493
			80	195.1	08.71 ± 0.237	57.25 ± 0.730
			100	243.9	05.33 ± 0.193	37.65 ± 0.387
Piperazine adipate	–	–	100	431.03	06.25 ± 0.062	24.50 ± 1.057



Comp. No.	R1	R2
2a	$-CH_3$	--
2b	$-C_3H_7$	--
3a	$-CH_3$	--
3b	$-C_3H_7$	--
4a	$-CH_3$	--
4b	$-C_3H_7$	--
5a	$-CH_3$	H
5b	$-CH_3$	*p*-OH
5c	$-CH_3$	*p*-NO_2
5d	$-CH_3$	*m*-NO_2
5e	$-CH_3$	*p*-Cl
5f	$-CH_3$	*o*-Cl
5g	$-CH_3$	*p*-OCH_3
5h	$-CH_3$	*m,p-di*-OCH_3
5i	$-C_3H_7$	H
5j	$-C_3H_7$	*p*-OH
5k	$-C_3H_7$	*p*-NO_2
5l	$-C_3H_7$	*m*-NO_2
5m	$-C_3H_7$	o-NO_2
5n	$-C_3H_7$	*p*-Cl
5o	$-C_3H_7$	*o*-Cl
5p	$-C_3H_7$	*m,p-di*-OCH_3

Fig. 1 Synthesis of (*E*)-2-methyl/propyl-4-[(substitutedbenzylidene) hydrazinyl]-5,6,7,8-tetrahydrobenzo[4,5]theino[2,3-*d*]pyrimidines (**5a**–**p**). Reagents and conditions: (i) RCN, dry HCl gas 6 h; (ii) $POCl_3$, 110 °C, 2–8 h (iii) $N_2H_4{\cdot}H_2O$, C_2H_5OH, 80 °C; (iv) R2-C_6H_4CHO, stirring room temperature

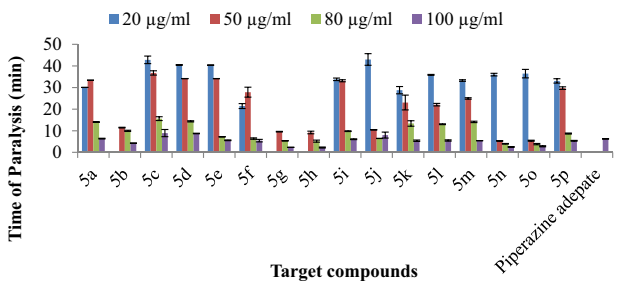


Fig. 2 Time of paralysis of target compounds 5a–p. Each value is expressed as mean ($n = 6$) ± SEM

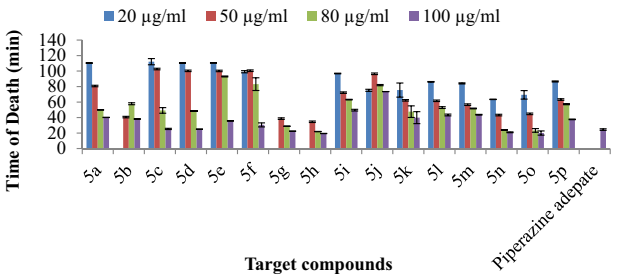


Fig. 3 Time of death of target compounds **5a**–**p**. Each value is expressed as mean ($n = 6$) ± SEM

the worms in 4.24, 5.58, 5.43, 2.32, 2.22, 5.39, 5.49, 5.41, 2.5, 2.81 and 5.33 min, respectively; whereas at 80 µg/ml (207.8–227.3 µM) concentration, compounds **5g**, **5h**, **5n**, **5o** paralyzed the worms in a mean time of 5.35, 5.18, 3.98 and 3.81 min, respectively. Similarly, at 100 µg/ml (431.03 µM) mean lethal time for the standard drug and compounds **5g**, **5h**, **5n**, **50** (284.0, 261.8, 259.7, and 259.7 µM respectively) were found to be 24.5, 22.38, 19.43, 21 and 20.03 min, respectively. The *t*-test was run for all the compounds using graph pad prism. Compounds **5b**, **5d**, **5e**, **5g**, **5h**, **5j**, **5l**, **5m**, **5n**, **5o**, and **5p** have shown significant variation in inducing paralysis (p value < 0.05) at all the test concentrations and the compounds **5a**, **5b**, **5e**, **5i**, **5j**, **5l**, **5m**, **5n**, **5o**, and **5p** have shown significant helminthicidal activity (p value < 0.05) at all the test concentrations when compared with that of the standard drug (100 µg/ml, 431.03 µM). At 50 µg/ml of test concentration, all the drugs have shown moderate paralytic as well as helminthicidal activity when compared with that of the standard. Finally, it can be concluded from the results that the compounds **5g**, **5h**, **5n**, **5o** have shown potent anthelminthic activity at 100 µg/ml (284.0, 261.8, 259.7, and 259.7 µM, respectively) with a mean paralytic time of 2.32, 2.22, 2.5, 2.81 min and helminthicidal time of 22.38, 19.43, 21, 20.03 min, respectively, when compared with that of the standard (6.25 and 24.5 min time for paralysis and death) as shown in Figs. 2 and 3.

Prediction of ADME properties and drug-likeness

The ADME (absorption, distribution, metabolism, and excretion) parameters like physicochemical properties, lipophilicity, water solubility, pharmacokinetics, and drug-like nature of the molecules (**5a–p**) were tested employing SwissADME software (http://swissadme.ch/) (Daina et al. 2017). The details of the calculated parameters are presented in Table 2. Except compounds **5n** and **5o**, all the compounds satisfied the

Table 2 Predicted physicochemical properties, pharmacokinetics and drug-likeness of (*E*)-2-methyl/propyl-4-(2-(substitutedbenzylidene) hydrazinyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidines (5a–p) and piperazine adipate

Molecule	Piperazine adipate	5a	5b	5c	5d	5e	5f	5g	5h	5i	5j	5k	5l	5m	5n	5o	5p
MW	232.28	322.4	338.4	367.4	367.4	356.8	356.8	352.4	382.4	350.4	366.4	395.4	395.4	395.4	384.9	384.9	410.5
RB	5	3	3	4	4	3	3	4	5	5	5	6	6	6	5	5	7
HBA	6	3	4	5	5	3	3	4	5	3	4	5	5	5	3	3	5
HBD	4	1	2	1	1	1	1	1	1	1	2	1	1	1	1	1	1
MR	67.16	97.3	99.3	106.1	106.1	102.3	102.3	103.8	110.3	106.9	108.9	115.7	115.7	115.7	111.9	111.9	119.9
F-Csp^3	0.8	0.28	0.28	0.28	0.28	0.28	0.28	0.32	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.41
TPSA	98.66	78.4	98.6	124.2	124.2	78.4	78.4	87.6	96.9	78.4	98.6	124.2	124.2	124.2	78.4	78.4	96.9
iLOGP	1.67	3.25	2.84	2.82	2.78	3.41	3.48	3.3	3.59	3.63	3.28	3.29	3.23	3.32	3.85	3.96	4.08
XLOGP3	−5.34	5.13	4.78	4.96	4.96	5.76	5.76	5.1	5.08	5.92	5.57	5.75	5.75	5.75	6.55	6.55	5.87
Log S	HS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	PS	PS	PS
GIA	High	High	High	High	High	High	High	High	High	High	High	Low	Low	Low	High	High	High
BBB-P	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Pgp-S	No	No	No	No	No	No	No	No	No	No	No	No	No	No	Yes	Yes	No
CYP1A2i	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	No
CYP2C9i	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
CYP2D6i	No	No	No	No	No	No	No	No	Yes	No	Yes	No	No	No	No	No	Yes
CYP3A4i	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Lipinski violations	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
Ghose violations	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BS	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55
PAINS-A	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0

MW molecular weight, *RB* rotatable bonds, *HBA* H-bond acceptor, *HBD*: H-bond donor, *MR* molar refractivity, *F-Csp*^{3} fraction Csp^3 to identify the saturation, iLOGP, *HS* highly soluble, *MS* moderately soluble, *PS* poorly soluble, *GIA* GI absorption, *BBB-P* BBB permeant, *Pgp-S* Pgp substrate, *i* inhibitor for all CYP, *BS* bioavailability score, *PAINS-A* Pan Assay interference structure alerts

Lipinski's rule of five (5). On the contrary, all molecules successfully passed the Ghose drug-like filter. To display the drug-likeness of the identified most active molecules according to anthelmintic assay and activity evaluation, six physicochemical properties have been taken into consideration: lipophilicity, size, polarity, solubility, flexibility, and saturation (Expressed as Lipo, Size, Polar, Insolu, Flex, and Insatu, respectively in Fig. 4), generated Bioavailability Radar plots. A physicochemical range on each axis was defined by descriptors and the pink area in which the radar plot of the compound has to stay completely to be identified as a drug-like molecule. Here, the pink prepared with the optimum range for individual properties (size: MW between 150 and 500 g/mol, polarity: TPSA between 20 and 130 Å^2, solubility: lipophilicity: XLOGP3 between −0.7 and +5.0, log *S* not higher than 6, flexibility: no more than 9 rotatable bonds and saturation: the fraction of carbons in the sp^3 hybridization not less than 0.25. It can be evident from the plots in Fig. 4 that in case of the most active compounds (**5g**, **5h**, **5n**, and **5o**), major drug-likeness properties are staying within the acceptable specified pink area which make them probable future lead molecules for anthelmintic activity. To compare the predicted parameters of the synthesized compounds with the standard drug, we have predicted the similar features for piperazine adipate. Interestingly, we found almost similar bioavailability score for piperazine adipate and all the synthesized compounds (**5a–p**). The predicted parameters strongly support the obtained outcomes from the anthelmintic assay and activity of the synthesized compounds (**5a–p**).

Conclusion

A series of some novel (*E*)-2-methyl/propyl-4-(2-(substitutedbenzylidene)hydrazinyl)-5,6,7,8-tetrahydrobenzo [4,5]thieno[2,3-*d*]pyrimidines was synthesized and characterized. Two different series of compounds having a methyl (**5a–h**) and propyl (**5i–p**) substituents at the 2-position of the thieno[2,3-*d*]pyrimidine scaffold with different electron withdrawing and electron donating groups at the aromatic ring were evaluated for their preliminary anthelmintic activity against adult Indian earthworms (*P.*

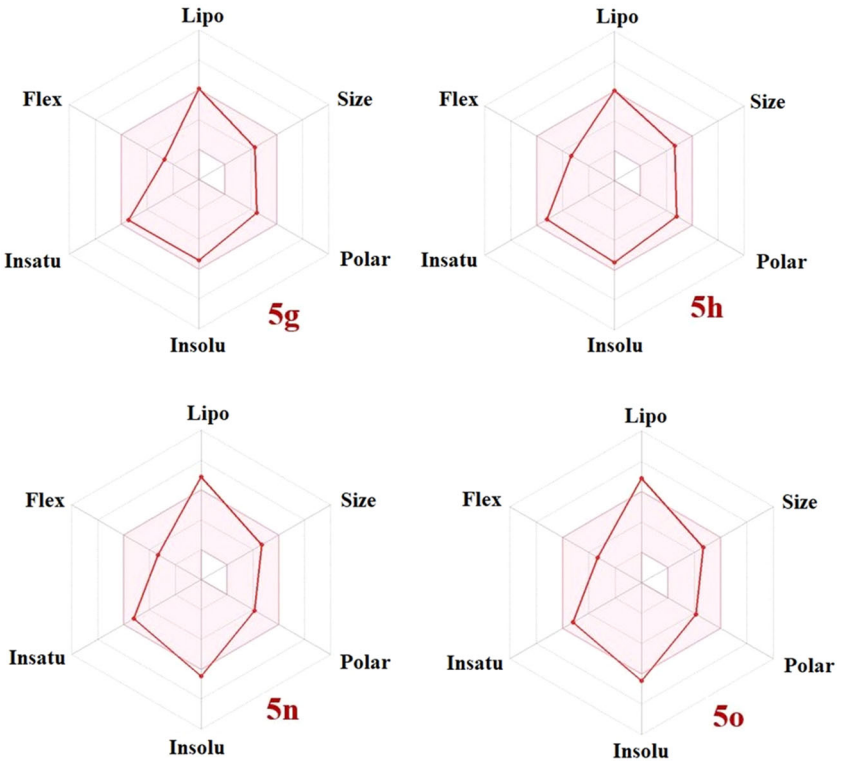


Fig. 4 Bioavailability Radar plots of the most active compounds **5g**, **5h**, **5n**, and **5o**

posthuma). Interestingly, compounds **5g** and **5h** having methyl group at the 2-position of the scaffold with electron donating methoxy group at *para*-position and dimethoxy group at *meta* and *para*-positions of the aromatic ring showed potent anthelmintic activity at 100 µg/ml (284.0 and 261.8 µM) with a mean paralytic time of 2.32, 2.22 min, and helminthicidal time of 22.38, 19.43 min, respectively. In contrast, the presence of electron withdrawing chloro group at *ortho* and *para*-position of the aromatic ring was found to be favorable for the anthelminthic activity of the compounds **5n** and **5o** with propyl group at the 2-position of the thieno[2,3-*d*]pyrimidine scaffold, exhibiting mean paralytic time of 2.5, 2.81 min and helminthicidal time of 21, 20.03 min, respectively, at the same concentration (100 µg/ml, 259.7 µM). Anthelmintic activities of these four compounds **5g**, **5h**, **5n**, and **5o** were found to be on par with the standard drug piperazine adipate (100 µg/ml, 431.03 µM), that showed the time for paralysis and death at 6.25, and 24.5 min, respectively. Overall, the potency of these compounds (**5g**, **5h**, **5n**, and **5o**) is better than standard drug as they exhibited the same activity at 259.7–284 µM as that of the standard drug (which has shown the same activity at 431.03 µM). The experimental outcomes are strongly supported by their satisfactory ADME and drug-like properties as predicated by the SwissADME software. Thus, further modification of these compounds might lead to the discovery of more potent analogs.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval We declare that all the co-authors are aware of and approve of the submission.

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