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Research Article

Synthesis and Evaluation of a New Series of 8-(2-Nitroaryl)Xanthines as Adenosine Receptor Ligands

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ABSTRACT A new series of 1,3-dimethylxanthine derivatives bearing 8-(2-nitroaryl) residue was synthesized and evaluated for affinity for recombinant human adenosine receptors subtypes. Nitrate esters of 7-substituted-1,3-dimethyl-8-phenylxanthines were also synthesized and tested. Introducing a nitro substituent at the 2-position of the 8-substituted phenyl ring resulted in generally low affinity for adenosine receptors (ARs), selectivity toward the A_{2A} subtype was enhanced in some of the compounds. 8-(4-Cyclopentyloxy-5-methoxy-2-nitrophenyl)-1,3-dimethylxanthine (**9e**) proved to be a potent compound among the 2-nitrophenyl substituted xanthines exhibiting a $K_i = 1$ μM at human A_{2A} ARs with at least 30 fold selectivity versus human A_1 and A_{2B} ARs. Replacement of 8-chloropropoxy phenyl with 8-nitrooxypropoxy phenyl resulted in a negligible change in binding affinity of the 8-substituted xanthines for various AR subtypes. Drug Dev Res 00 : 000–000, 2016.

Key words: nitroxanthines; adenosine receptors; nitrate esters

INTRODUCTION

Adenosine, an endogeneous nucleoside present ubiquitously in all mammalian cells, modulates a variety of physiological functions via G protein-coupled adenosine receptors, classified as A_1, A_{2A}, A_{2B}, and A_3 subtypes. For each subtype, selective agonists, and antagonists have been developed as potential new drug candidates for the treatment of various ailments such as neurodegenerative, respiratory, cardiac, immune, and inflammatory disorders [Muller and Jacobson, 2011; Chen et al., 2013; Layland et al., 2014]. The development of potent and selective ligands for adenosine receptors (ARs) has been a dynamic area of research for several decades.

Substituted xanthines represent a privileged class of pharmacologically active compounds with well-known binding affinity for ARs. Xanthines have been widely explored in terms of affinity and selectivity for ARs [Daly, 2000; Bansal et al., 2009; Yadav et al., 2014]. Introduction of 8-aryl/heteroaryl substituents in the xanthine scaffold results in spectacular alterations in their affinity for ARs [Baziard-

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Mouysset et al., 1995]. Several 8-(substituted phenyl) derivatives are potent AR ligands [Daly et al., 1986]. It has been observed that the substitution pattern of 8-phenyl substituents dramatically affects the AR binding affinity. 8-(4-Nitrophenyl)-1,3-dimethylxanthine (**1**) (Fig. 1) has been reported as a potent A_1 AR antagonist with an IC_{50} value of 8 nM in competition for N^6-[^{3}H] cyclohexyladenosine binding at bovine brain membranes [Hamilton et al., 1985]. We decided to synthesize a new series of 8-nitroarylxanthines and study their binding affinity for ARs to gain further insight into the structure-activity relationship of xanthines at ARs. In view of the significance of nitrate esters (**2**) to display a variety of biological actions due to their ability to generate nitric oxide [Lagas and Duchateau, 1988; Kerwin et al., 1995], it was further planned to synthesize and study the effect of introducing nitrate esters on adenosine binding affinity of xanthines.

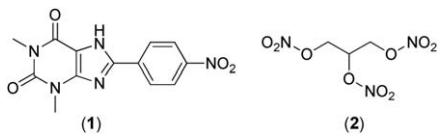


Fig. 1. Structures of nitroxanthine (**1**) and nitrate ester (**2**).

METHODS AND MATERIALS

Chemistry

All melting points were taken on a Veego melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on Perkin-Elmer 882 and RX1 FT-IR spectrophotometers as potassium bromide pellets (υ_{max} in cm^{-1}). Proton (^{1}H) nuclear magnetic resonance spectroscopy was performed using a Brucker AC-300F, 300 MHz spectrometer for solutions in deuteriochloroform, deuterated dimethylsulfoxide and are reported in parts per million (ppm). Tetramethylsilane (Me_4Si) was used as an internal reference. The spin multiplicities are designated by the symbols, s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), m (multiplet), and br (broad). Elemental analyses were carried out on a Perkin-Elmer-2400 CHN elemental analyzer. Plates for thin layer chromatography (TLC) were prepared with silica gel G according to method of Stahl (E. Merck) using ethyl acetate as solvent and activated at 110°C for 30 min. Iodine was used to develop the TLC plates. Anhydrous sodium sulfate was utilized as drying agent. All solvents were freshly distilled and dried prior to use according to standard procedures. Nitroaldehyde **7a** was prepared by a reported procedure [Bogert and Elder, 1929]. 7-Substituted xanthine derivatives **17**, **18**, and **22** were also synthesized as described [Bansal et al., 2011].

General Method for the Synthesis of Nitroaldehydes 7b–e

Requisite aromatic aldehyde 3,4-dimethoxybenzaldehyde, 4-methoxybenzaldehyde, 3,4,5-trimethoxybenzaldehyde, 4-cyclopentyloxy-3methoxybenzaldehyde (1 g) was added in small portions to the stirred ice cold mixture of concentrated nitric acid (3 ml) and concentrated sulfuric acid (2 ml). The reaction mixture was further stirred in ice for 20–25 min and then at room temperature for 15 min. The reaction mixture was poured onto crushed ice. The yellow colored precipitate obtained was filtered off, washed with ice cold water, dried, and crystallized from ethanol to obtain the corresponding nitroaldehydes **7b–e**.

4,5-Dimethoxy-2-nitrobenzaldehyde (**7b**). Yield: (0.9 g, 70.86%), m.p. 126–128°C. ^{1}H NMR ($CDCl_3$): δ 4.15 (s, 6H, 2 X —OC*H*$_3$), 7.45 (s, 1H, C*H*, aromatic), 7.65 (s, 1H, C*H*, aromatic), and 10.25 ppm (s, 1H, —C*H*O).

4-Methoxy-2-nitrobenzaldehyde (**7c**). Yield: (0.98 g, 74.0%), m.p. 76–80°C. ^{1}H NMR ($CDCl_3$): δ 4.05 (s, 3H, —OC*H*$_3$), 7.30 (d, 1H, C*H*, aromatic, $J_o = 7.80$ Hz), 8.15 (dd, 1H, C*H*, aromatic, $J_o = 8.0$, $J_m = 2.0$ Hz), 8.35 (d, 1H, C*H*, aromatic, $J_m = 2.0$ Hz), and 10.35 ppm (s, 3H, —C*H*O).

3,4,5-Trimethoxy-2-nitrobenzaldehyde (**7d**). Yield: (0.6 g, 48.78%), m.p. 110–112°C. ^{1}H NMR ($CDCl_3$): δ 4.05 (s, 9H, 3 X —OC*H*$_3$), 7.30 (s, 1H, C*H*, aromatic), and 9.90 ppm (s, 1H, —C*H*O).

4-Cyclopentyloxy-5-methoxy-2-nitrobenzaldehyde (**7e**). Yield: (0.67 g, 55.83%), m.p. 80–84°C. ^{1}H NMR ($CDCl_3$): δ 1.80 (br s, 8H, 4 X C*H*$_2$, cyclopentyl), 4.05 (s, 3H, —OC*H*$_3$), 4.95 (s, 1H, O—C*H*<, cyclopentyl), 7.40 (m, 1H, C*H*, aromatic), 7.66 (s, 1H, C*H*, aromatic), and 10.55 ppm (s, 1H, —C*H*O).

General Method for the Synthesis of Substituted Aldehydes 10a–g

The respective hydrochlorides of β-dialkylaminoethyl chloride (6.57 mmol), 1-bromo-3-chloropropane (1.5 ml, in excess)/1-bromo-2-chloroethane (1.5 ml, in excess) were added to a stirred and refluxing slurry of vanillin (1.0 g, 6.57 mmol) in ethyl methyl ketone (40 ml) in the presence of anhydrous potassium carbonate (2.0 g, 14.47 mmol) as reported earlier [Bansal et al., 2009, 2011]. The reaction mixture was further refluxed for 6 h with continuous stirring. The completion of the reaction was monitored by TLC. On completion, the reaction mixture was cooled, filtered and the solvent was removed under

reduced pressure to obtain an oily residue of the corresponding substituted aldehyde **10a–g**, which was used as such for further reaction.

General Method for the Synthesis of Nitroaldehydes 11a–g

The above obtained substituted aldehyde **10a–g** (oily residue) was added in small portions to the stirred ice cold mixture of concentrated nitric acid (3 ml) and concentrated sulfuric acid (2 ml). The reaction mixture was further stirred in ice for 20–25 min and then at room temperature for 15 min and then poured onto crushed ice. The yellow colored precipitate obtained was filtered off, washed with ice cold water, dried and crystallized from ethanol to obtain corresponding compound **11a–g**.

4-(2-Dimethylaminoethoxy)-5-methoxy-2-nitrobenzaldehyde (**11a**). Yield: (0.35 g, 29.16%), m.p. 60–64°C. ^{1}H NMR ($CDCl_3$): δ 2.43 (*t*, 6H, —N(CH_3)$_2$), 2.84 (*t*, 2H, —CH_2N<, $J = 5.67$ Hz), 3.99 (s, 3H, —OCH_3), 4.24 (*t*, 2H, —OCH_2—, $J = 5.74$ Hz), 7.40 (s, 1H, C*H*, aromatic), 7.64 (s, 1H, C*H*, aromatic), and 10.44 ppm (s, 1H, —C*H*O).

4-(2-Diethylaminoethoxy)-5-methoxy-2-nitrobenzaldehyde (**11b**). Yield: (0.38 g, 32.47%), m.p 72–76°C. ^{1}H NMR ($CDCl_3$): δ 1.09 (*t*, 6H, —N(CH_2CH_3)$_2$, $J = 7.03$ Hz), 2.68 (m, 4H, —N(CH_2CH_3)$_2$), 2.99 (*t*, 2H, —CH_2N<, $J = 5.7$ Hz), 4.00 (s, 3H, —OCH_3), 4.21 (*t*, 2H, —OCH_2—, $J = 5.6$ Hz), 7.01 (s, 1H, C*H*, aromatic), 7.66 (s, 1H, C*H*, aromatic), and 9.84 ppm (s, 1H, —C*H*O).

3-Methoxy-2-nitro-4-(2-piperidin-1-ylethoxy)benzaldehyde (**11c**). Yield: (0.25 g, 21.36%), m.p 110–114°C. ^{1}H NMR ($CDCl_3$): δ 1.47 (m, 2H, —CH_2, piperidine), 1.62 (m, 4H, 2 X CH_2, piperidine), 2.54 (s, 4H, —N(CH_2)$_2$, piperidine), 2.86 (*t*, 2H, —CH_2N<, $J = 6.10$ Hz), 4.00 (s, 3H, —OCH_3), 4.27 (*t*, 2H, —OCH_2—, $J = 6.12$ Hz), 7.40 (s, 1H, C*H*, aromatic), 7.68 (s, 1H, C*H*, aromatic), and 10.44 ppm (s, 1H, —C*H*O).

5-Methoxy-4-(2-morpholin-4-ylethoxy)-2-nitrobenzaldehyde (**11d**). Yield: (0.4 g, 34.48%), m.p 98–102°C. ^{1}H NMR ($CDCl_3$): δ 2.61 (m, 4H, —N(CH_2)$_2$, morpholine), 2.90 (*t*, 2H, —CH_2N<, $J = 5.77$ Hz), 3.74 (m, 4H, O(CH_2)$_2$, morpholine), 4.00 (s, 3H, —OCH_3), 4.29 (*t*, 2H, —OCH_2—, $J = 5.83$ Hz), 7.42 (s, 1H, C*H*, aromatic), 7.67 (s, 1H, C*H*, aromatic), and 10.45 ppm (s,1H, —C*H*O).

5-Methoxy-2-nitro-4-(2-pyrrolidin-1-ylethoxy)benzaldehyde (**11e**).Yield: (0.38 g, 32.20%), m.p 122–126°C. ^{1}H NMR ($CDCl_3$): δ 1.83 (m, 4H, 2 X CH_2, pyrrolidine), 2.68 (m, 4H, —N(CH_2)$_2$, pyrrolidine), 3.02 (*t*, 2H, —CH_2N<, $J = 5.94$ Hz), 4.00 (s, 3H, —OCH_3), 4.29 (*t*, 2H, —OCH_2—, $J = 5.91$ Hz), 7.40 (s, 1H, C*H*, aromatic), 7.65 (s, 1H, C*H*, aromatic), and 10.44 ppm (s, 1H, —C*H*O).

4-(2-Chloropropoxy)-3-methoxy-2-nitrobenzaldehyde (**11f**). Yield: (1.1 g, 92.43%), m.p 76–80°C. ^{1}H NMR ($CDCl_3$): δ 2.35 (m, 2H, —CH_2CH_2—), 3.78 (*t*, 2H, —CH_2Cl, $J = 6.0$ Hz), 4.05 (s, 3H, —OCH_3), 4.25 (*t*, 2H, —OCH_2—, $J = 6.0$ Hz), 7.45 (s, 1H, C*H*, aromatic), 7.55 (s, 1H, C*H*, aromatic), and 10.25 ppm (s, 1H, —C*H*O).

4-(2-Chloroethoxy)-3-methoxy-2-nitrobenzaldehyde (**11g**). Yield: (0.58 g, 48.33%), m.p 158–160°C. ^{1}H NMR ($CDCl_3$): δ 3.93 (t, 2H, —CH_2Cl, $J = 6.36$ Hz), 4.02 (s, 3H, —OCH_3), 4.42 (*t*, 2H, —OCH_2—, $J = 5.71$ Hz), 7.43 (s, 1H, C*H*, aromatic), 7.63 (s, 1H, C*H*, aromatic), and 10.45 ppm (s, 1H, —C*H*O).

General Method for the Synthesis of Benzylidene Derivatives 8a–e, 12a–g

To a stirred solution of 5,6-diamino-1,3-dimethyluracil (6, 1.0 g, 5.87 mmol) in MeOH–AcOH (4:1, 40 ml) was slowly added the requisite nitroaldehyde **7a–e** and **11a–g** (1.0 g) in methanol (24 ml). Yellow colored precipitate appeared during addition. The reaction mixture was further stirred overnight at room temperature and the completion of reaction was monitored by TLC. The precipitate obtained was filtered off, washed with methanol and dried to obtain corresponding quite unstable benzylidene derivatives **8a–e** and **12a–g**, which were used as such for further cyclization. The physicochemical data of the intermediates **8a–e** and **12a–g** are presented in Table 1.

General Method for Synthesis of Various Xanthine Derivatives 9a–e and 13a–g

Benzylidene derivatives **8a–e** and **12a–g** (1.0 g, 2 mmol) thus obtained were cyclized individually by refluxing in thionyl chloride (20 ml) for 30–40 min. The excess thionyl chloride was removed under reduced pressure to get a solid product. Ice cold water was added to it and resulting suspension was neutralized with ammonium hydroxide solution. The precipitate obtained was collected by filtration, dried and recrystallized from ethanol to afford the desired products **9a–e** and **13a–g**, respectively.

1,3-Dimethyl-8-(6-nitrobenzo[1,3]dioxol-5-yl)xanthine (**9a**). Yield: (0.94 g, 94.56%), m.p. 318–322°C. FTIRυ_{max} (KBr): 3040, 2920, 1695, 1640, 1490, 1360, 1240, and 1005 cm^{-1}. ^{1}H NMR ($CDCl_3$-DMSO-d_6): δ 3.35 (s, 3H, N—CH_3), 3.54 (s, 3H, N—CH_3), 6.23 (s, 2H, —O—CH_2—O—), 7.18 (s, 1H, C*H*, aromatic), 7.94 (s, 1H, C*H*, aromatic), and 13.74 ppm (br s, 1H, N—*H*).

TABLE 1. Physicochemical Data of Benzylidene Derivatives 8a–e and 12a–g

Comp no	Yield (%)	m.p. (°C)	Comp no	Yield	m.p. (°C)
8a	44.11	260–264	**12b**	33.33	208–214
8b	58.68	258–262	**12c**	34.35	214–218
8c	63.07	>300	**12d**	35.46	160–164
8d	26.83	160–170	**12e**	27.57	202–208
8e	34.69	238–242	**12f**	36.92	200–204
12a	52.8	200–204	**12g**	47.61	234–238

Calcd. for $C_{14}H_{11}N_5O_6$: C, 48.70; H, 3.21; N, 20.28%. Found: C, 48.31; H, 2.92; N, 20.32%.

8-(4,5-Dimethoxy-2-nitrophenyl)-1,3-dimethylxanthine (**9b**). Yield: (0.73 g, 72.94%), m.p. 304–308°C. FTIRυ_{max} (KBr): 3158, 2920, 1704, 1652, 1546, 1475, 1351, 1281, and 1011 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.33 (s, 3H, N—CH_3), 3.67 (s, 3H, N—CH_3), 4.03 (s, 6H, 2 X —OCH_3), 7.25 (s, 1H, C*H*, aromatic), 7.70 (s, 1H, C*H*, aromatic), and 13.10 ppm (br s, 1H, N—*H*). Calcd. for $C_{15}H_{15}N_5O_6$: C, 49.86; H, 4.18; N, 19.38%. Found: C, 49.34; H, 3.90; N, 18.88%.

1,3-Dimethyl-8-(4-methoxy-2-nitrophenyl)xanthine (**9c**). Yield: (0.7g, 70.49%), m.p. >300°C. FTIRυ_{max} (KBr): 2941, 1709, 1662, 1522, 1333, 1223, and 1052 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.41 (s, 3H, N—CH_3), 3.63 (s, 3H, N—CH_3), 4.04 (s, 3H, —OCH_3), 7.23 (d, 1H, C*H*, aromatic, J_o = 8.93 Hz), 8.41 (dd, 1H, C*H*, aromatic, J_o = 8.73, J_m = 2.14 Hz), and 8.75 ppm (d, 1H, C*H*, aromatic, J_m = 2.12 Hz). Calcd. for $C_{14}H_{13}N_5O_5$: C, 50.76; H, 3.95; N, 21.14%. Found: C, 50.45; H, 3.85; N, 20.81%.

1,3-Dimethyl-8-(3,4,5-trimethoxy-2-nitrophenyl) xanthine (**9d**). Yield: (0.33 g, 47.4%), m.p. 256–260°C. FTIRυ_{max} (KBr): 3107, 2949, 1699, 1642, 1606, 1577, 1542, 1476, 1375, 1197, and 1027 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.39 (s, 3H, N—CH_3), 3.62 (s, 3H, N—CH_3), 3.98 (s, 3H, —OCH_3), 3.99 (s, 3H, —OCH_3), 4.02 (s, 3H, —OCH_3), 7.22 (s, 1H, C*H*, aromatic), and 12.55 ppm (br s, 1H, N—*H*). Calcd. for $C_{16}H_{17}N_5O_7$: C, 49.11; H, 4.38; N, 17.89%. Found: C, 48.78; H, 3.87; N, 17.39%.

8-(4-Cyclopentyloxy-5-methoxy-2-nitrophenyl)-1,3-dimethylxanthine (**9e**). Yield: (0.17 g, 24.13%), m.p. 220–224°C. FTIRυ_{max} (KBr): 2940, 1710, 1640, 1580, 1520, 1335, 1280, 1220, and 1050 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.71 (m, 2H, CH_2, cyclopentyl), 1.88 (m, 4H, 2 X CH_2, cyclopentyl), 2.06 (m, 2H, CH_2, cyclopentyl), 3.31 (s, 3H, N—CH_3), 3.68 (s, 3H, N—CH_3), 3.99 (s, 2H, —OCH_3), 4.92 (m, 1H, O—C*H*<, cyclopentyl), 7.19 (s, 1H, C*H*, aromatic), 7.70 (s, 1H, C*H*, aromatic), and 13.30 ppm (br s, 1H, N—*H*). Calcd. for $C_{19}H_{21}N_5O_6$: C, 54.94; H, 5.09; N, 16.86%. Found: C, 54.76; H, 4.91; N, 16.44%.

8-[4-(2-Dimethylaminoethoxy)-5-methoxy-2-nitrophenyl]-1,3-dimethylxanthine (**13a**). Yield: (0.1 g, 25.12%), m.p. 148–152°C. FTIRυ_{max} (KBr): 2920, 1710, 1650, 1510, 1340, 1220, and 1050 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.43 (*t*, 6H, —N(CH_3)$_2$), 2.91 (*t*, 2H, —CH_2N<, J = 5.45 Hz), 3.33 (s, 3H, N—CH_3), 3.66 (s, 3H, N—CH_3), 3.98 (s, 3H, —OCH_3), 4.26 (*t*, 2H, —OCH_2—, J = 5.54 Hz), 7.24 (s, 1H, C*H*, aromatic), and 7.71 ppm (s, 1H, C*H*, aromatic). Calcd. for $C_{18}H_{22}N_6O_6$: C, 51.67; H, 5.30; N, 20.09%. Found: C, 50.91; H, 4.98; N, 19.81%.

8-[4-(2-Diethylaminoethoxy)-5-methoxy-2-nitrophenyl]-1,3-dimethylxanthine (**13b**). Yield: (0.12 g, 20.33%), m.p. 132–136°C. FTIRυ_{max} (KBr): 2974, 1708,1655, 1509, 1335, 1220, and 1051 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.09 (*t*, 6H, —N(CH_2CH_3)$_2$, J = 7.12 Hz), 2.67 (m, 4H, —N(CH_2CH$_3$)$_2$), 2.98 (*t*, 2H, —CH_2N<, J = 6.50 Hz), 3.32 (s, 3H, N—CH_3), 3.66 (s, 3H, N—CH_3), 3.99 (s, 3H, —OCH_3), 4.21 (*t*,2H, —OCH_2—, J = 6.25 Hz), 7.21 (s, 1H, C*H*, aromatic), and 7.74 ppm (s, 1H, C*H*, aromatic). Calcd. for $C_{20}H_{26}N_6O_6$: C, 53.80; H, 5.87; N, 18.82%. Found: C, 53.67; H, 5.66; N, 18.21%.

1,3-Dimethyl-8-[5-methoxy-2-nitro-4-(2-piperidin-1-ylethoxy)phenyl]xanthine (**13c**). Yield: (0.3 g, 43.10%), m.p. 142–146°C. FTIRυ_{max} (KBr): 2941, 2793, 1709, 1652, 1503, 1334, 1221, and 1048 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.48 (m, 2H, —CH_2, piperidine), 1.63 (p, 4H, 2 X CH_2, piperidine), 2.55 (s, 4H, —N(CH_2)$_2$, piperidine), 2.87 (*t*, 2H, —CH_2N<, J = 6.01 Hz), 3.34 (s, 3H, N—CH_3), 3.66 (s, 3H, N—CH_3), 3.99 (s, 3H, —OCH_3), 4.27 (*t*, 2H, —OCH_2—, J = 5.96 Hz), 7.23 (s, 1H, C*H*, aromatic) and 7.74 ppm (s, 1H, C*H*, aromatic). Calcd. for $C_{21}H_{26}N_6O_6$: C, 55.01; H, 5.72; N, 18.33%. Found: C, 54.78; H, 5.37; N, 18.13%.

1,3-Dimethyl-8-[5-methoxy-4-(2-morpholin-4-ylethoxy)-2-nitrophenyl]xanthine (**13d**). Yield: (0.08 g, 8.08%), m.p. 198–202°C. FTIRυ_{max} (KBr): 2920, 1710, 1655, 1510, 1335, 1270, 1220, and 1115 cm[1]. ^1H NMR ($CDCl_3$): δ 2.62 (*t*, 4H, —N(CH_2)$_2$, morpholine, J = 4.61 Hz), 2.91 (*t*, 2H, —CH_2N<, J = 5.72 Hz), 3.33 (s, 3H, N—CH_3), 3.66 (s, 3H, N—CH_3), 3.74 (*t*, 4H, O(CH_2)$_2$, morpholine, J = 4.65 Hz), 4.00 (s, 3H, —OCH_3), 4.26 (*t*, 2H, —OCH_2—, J = 5.79 Hz), 7.25 (s, 1H, C*H*, aromatic), and 7.74 ppm (s, 1H, C*H*, aromatic). Calcd. for $C_{20}H_{24}N_6O_7$: C, 52.17; H, 5.25; N, 18.25%. Found: C, 51.88; H, 4.98; N, 17.93%.

1,3-Dimethyl-8-[5-methoxy-2-nitro-4-(2-pyrrolidin-1-ylethoxy)phenyl]xanthine (**13e**). Yield: (0.26 g, 43.55%), m.p. 118–122°C. FTIRυ_{max} (KBr): 2925,

1677, 1630, 1522, 1349, 1211, and 1052 cm^{-1}.^{1}H NMR ($CDCl_3$): δ 1.63 (p, 4H, 2 X CH_2, pyrrolidine), 2.67 (m, 4H, —N(CH_2)$_2$, pyrrolidine), 3.04 (*t*, 2H, CH_2N<, J = 5.83Hz), 3.34 (s, 3H, N—CH_3), 3.66 (s, 3H, N—CH_3), 3.96 (s, 3H, —OCH_3), 4.28 (*t*, 2H, —OCH_2—, J = 5.84 Hz), 7.23 (s, 1H, C*H*, aromatic), and 7.71 ppm (s, 1H, C*H*, aromatic). Calcd. for $C_{20}H_{24}N_6O_6$: C, 54.05; H, 5.44; N, 18.91%. Found: C, 53.98; H, 5.25; N, 18.71%.

8-[4-(3-Chloropropoxy)-5-methoxy-2-nitrophenyl]-1,3-dimethylxanthine (**13f**). Yield: (0.75 g, 75.75%), m.p. 232–236°C. FTIRυ_{max} (KBr): 2930, 1695, 1660, 1550, 1335, 1270, 1220, and 1050 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 2.36 (p, 2H, —CH_2CH_2—), 3.32 (s, 3H, N—CH_3), 3.67 (s, 3H, N—CH_3), 3.80 (*t*, 2H, —CH_2Cl, J = 6.16 Hz), 4.01 (s, 3H, —OCH_3), 4.31 (*t*, 2H, —OCH_2—, J = 5.91 Hz), 7.23 (s, 1H, C*H*, aromatic), 7.73 (s, 1H, C*H*, aromatic), and 13.13 ppm (br s, 1H, N—*H*). Calcd. for $C_{17}H_{18}N_5O_6Cl$: C, 48.18; H, 4.28; N, 16.52%. Found: C, 47.97; H, 3.90; N, 16.20%.

8-[4-(2-Chloroethoxy)-5-methoxy-2-nitrophenyl]-1,3-dimethylxanthine (**13g**). Yield: (0.56 g, 56.28%), m.p. 286–290°C. FTIRυ_{max} (KBr): 3116, 1701, 1658, 1571, 1531, 1485, 1379, 1282, 1215, and 1023 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 3.35 (s, 3H, N—CH_3), 3.67 (s, 3H, N—CH_3), 3.92 (*t*, 2H, —CH_2Cl, J = 5.63 Hz), 4.03 (s, 3H, —OCH_3), 4.41 (*t*, 2H, —OCH_2—, J = 5.77 Hz), 7.32 (s, 1H, C*H*, aromatic), and 7.72 ppm (s, 1H, C*H*, aromatic). Calcd. for $C_{16}H_{16}N_5O_6Cl$: C, 46.90; H, 3.94; N, 17.09%. Found: C, 46.77; H, 3.76; N, 16.94%.

General Procedure for the Synthesis of Xanthine Derivatives 14 and 15

A mixture of 8-[4-(3-chloropropoxy)]xanthine derivatives **13f** or **13g** (1.0 g) and imidazole (1.0 g, 14.68 mmol) was fused together at 120°C for 3 h. The fused reaction mixture was cooled, water was added to remove the unreacted imidazole and cooled in ice. The solid obtained was filtered off, washed with water, dried and recrystallized from a mixture of methanol and chloroform to obtain the corresponding imidazole derivatives **14** and **15**.

1,3-Dimethyl-8-[4-(3-imidazol-1-ylpropoxy)-5-methoxy-2-nitrophenyl]xanthine (**14**). Yield: (0.73 g, 67.91%), m.p. 238–242°C. FTIRυ_{max} (KBr): 2900, 1700, 1660, 1580, 1510, 1325, 1210, and 1040 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 2.33 (p, 2H, —CH_2CH_2—), 3.40 (s, 3H, N—CH_3), 3.59 (s, 3H, N—CH_3), 4.01 (s, 3H, —OCH_3), 4.07 (*t*, 2H, —CH_2N<, J = 5.82 Hz), 4.26 (*t*, 2H, —OCH_2—, J = 6.69 Hz), 7.02 (s, 2H, C*H*, imidazole), 7.29 (s, 1H, C*H*, aromatic), 7.51 (s, 1H, C*H*, aromatic), and 7.56 ppm (s, 1H, C*H*, imidazole). Calcd. for $C_{20}H_{21}N_7O_6$: C, 52.74; H, 4.65; N, 21.53%. Found: C, 52.60; H, 4.35; N, 21.23%.

1,3-Dimethyl-8-[4-(2-imidazol-1-ylethoxy)-5-methoxy-2-nitrophenyl]xanthine (**15**). Yield: (0.34 g, 45.09%), m.p. 260–264°C. FTIRυ_{max} (KBr): 2946, 1703, 1661, 1580, 1500, 1443, 1332, 1217, and 1050 cm^{-1}. ^{1}H NMR ($CDCl_3$-DMSO-d_6): δ 3.39 (s, 3H, N—CH_3), 3.57 (s, 3H, N—CH_3), 3.99 (s, 3H, —OCH_3), 4.41 (*t*, 2H, —CH_2N<, J = 4.72 Hz), 4.48 (*t*, 2H, —OCH_2—, J = 4.72 Hz), 7.02 (s, 1H, C*H*, imidazole), 7.21 (s, 1H, C*H*, imidazole), 7.29 (s, 1H, C*H*, aromatic), 7.55 (s, 1H, C*H*, aromatic), and 7.77 ppm (s, 1H, C*H*, imidazole). Calcd. for $C_{19}H_{19}N_7O_6$: C, 51.70; H, 4.34; N, 22.21%. Found: C, 51.63; H, 4.14; N, 22.18%.

General Procedure for the Synthesis of 8-Nitrooxypropoxyphenylxanthines 18, 19, and 21

A solution of silver nitrate (0.38 g, 2.23 mmol) in acetonitrile (4 ml) was added to stirred solution of desired xanthine derivative **16**, **17**, and **20** (0.50 g, 1.19 mmol) in sufficient amount of dry acetonitrile at 60°C. The reaction mixture was further stirred for 10 h while keeping the temperature constant. The solution was filtered off, filtrate was concentrated and water was added to it. The precipitate obtained was filtered off, washed with water, dried, and recrystallized from acetone to afford corresponding xanthine nitrate esters **18**, **19**, and **21**.

8-[3-Methoxy-4-(3-nitrooxypropoxy)phenyl-1,3,7-trimethylxanthine (**18**) (0.2 g, 37.45%), m.p. 122–124°C. FTIRυ_{max} (KBr): 2947, 1695, 1658, 1539, 1279, 1237, and 1028 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 2.31 (m, 2H, —CH_2CH_2—), 3.43 (s, 3H, N—CH_3), 3.63 (s, 3H, N—CH_3), 3.93 (s, 3H, N—CH_3), 4.05 (s, 3H, —OCH_3), 4.18 (*t*, 2H, —OCH_2—, J = 5.93 Hz), 4.72 (*t*, 2H, —CH_2ONO_2, J = 6.15 Hz), 6.99 (d, 1H, C*H*, aromatic, J_o = 8.25 Hz), 7.15 (dd, 1H, C*H*, aromatic, J_o = 8.13, J_m = 2.12 Hz), and 7.25 ppm (m, 1H, C*H*, aromatic). Calcd. for $C_{18}H_{21}N_5O_7$: C, 51.55; H, 5.05; N, 16.70%. Found: C, 51.21; H, 4.68; N, 16.23%.

1,3-Dimethyl-8-[3-methoxy-4-(3-nitrooxypropoxy) phenyl]-7-propylxanthine (**19**) (0.4 g, 75.47%), m.p. 150–154°C. FTIRυ_{max} (KBr): 2960, 1697, 1655, 1537, 1475, 1438, 1260, and 1031 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.88 (*t*, 3H, —CH_2CH_3, J = 7.37 Hz), 1.86 (m, 2H, —CH_2CH_3), 2.32 (m, 2H, —CH_2CH_2—), 3.44 (s, 3H, N—CH_3), 3.63 (s, 3H, N—CH_3), 3.92 (s, 3H, —OCH_3), 4.19 (*t*, 2H, —OCH_2—, J = 5.89 Hz), 4.30 (*t*, 2H, N—CH_2, J = 7.63 Hz), 4.72 (*t*, 2H, —CH_2ONO_2, J = 6.14 Hz), 6.98 (d, 1H, C*H*, aromatic, J_o = 8.06 Hz), and 7.15 ppm (m, 2H, C*H*, aromatic). Calcd. for

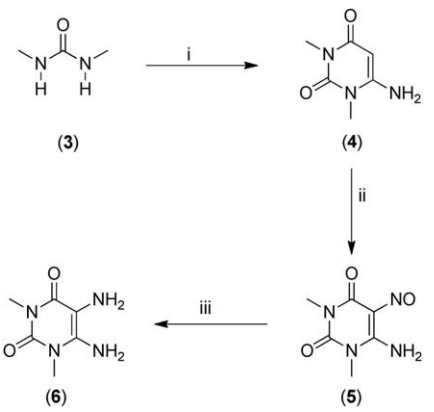


Fig. 2. Synthesis of 5,6-diamino-1,3-dimethyluracil (**6**). Reagents and reaction conditions: (i) cyanoacetic acid, acetic anhydride, reflux, sodium hydroxide; (ii) glacial acetic acid, sodium nitrite, ice bath; (iii) concentrated NH_4OH, sodium dithionite.

$C_{20}H_{25}N_5O_7$: C, 53.69; H, 5.63; N, 15.65%. Found: C, 53.43; H, 4.95; N, 15.39%.

1,3-Dimethyl-8-[3-(3-nitrooxypropoxy)phenyl]-7-propylxanthine (**21**) (0.23 g, 43.07%), m.p. 120–122°C. FTIRυ_{max} (KBr): 2961, 1697, 1660, 1540, 1281, 1224, and 1047 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.87 (*t*, 3H, —CH_2CH_3, $J = 7.36$ Hz), 1.84 (m, 2H, —CH_2CH_3), 2.26 (m, 2H, —CH_2CH_2—), 3.44 (s, 3H, N—CH_3), 3.63 (s, 3H, N—CH_3), 4.14 (*t*, 2H, —OCH_2—, $J = 5.85$ Hz), 4.32 (*t*, 2H, N—CH_2, $J = 7.52$ Hz), 4.69 (*t*, 2H, —CH_2ONO_2, $J = 6.21$ Hz), 7.05 (dd, 1H, C*H*, aromatic, $J_o = 8.42$, $J_m = 2.31$ Hz), 7.15 (m, 2H, C*H*, aromatic), and 7.43 ppm (*t*, 1H, C*H*, aromatic, $J_o = 7.96$ Hz). Calcd. for $C_{19}H_{23}N_5O_6$: C, 54.67; H, 5.55; N, 16.78%. Found: C, 53.81; H, 5.26; N, 16.36%.

Adenosine Receptor Binding Assays

The synthesized compounds were tested in radioligand binding assays using membranes from cells expressing cloned human adenosine A_1 (Euroscreen, Belgium) and A_{2A} receptors (Perkin Elmer, Beaconsfield, UK) based on the methods previously described [Bansal et al., 2009, 2011].

A_1 Receptor Binding

Basically, 10 mg human recombinant A_1 receptors were incubated with 20 nM [^{3}H]DPCPX (Tocris Cookson, Bristol, UK) in the presence or absence of compound in a Hepes (20 mM) buffer containing 100 mM NaCl and 100 mM $MgCl_2$ (Sigma Aldrich, Poole, UK). The assay was incubated at 25°C for 60 min in 96 well Multiscreen MHAF B3 H60 filter plates presoaked in 0.3% polyethyleneimine (PEI) (Millipore (Watford, UK). Total assay volume was 200 μl. Nonradioactive DPCPX (Tocris Cookson, Bristol, UK), in the concentration range of 1 nM to 3 μM, in half log units, was used as a standard to assess assay integrity.

A_{2A} Receptor Binding Assay

Twenty nanomolar [^{3}H]ZM241385 (Tocris Cookson, Bristol, UK) was incubated with 10 mg A_{2A} receptors in the presence or absence of compound in 50 mM Tris HCl buffer supplemented with 0.5mM EDTA and 10 mM $MgCl_2$ (Sigma Aldrich, Poole, UK). ZM241385 (Tocris Cookson, Bristol, UK), over the concentration range 0.1 pM-300 nM, was used as a standard compound in this assay system. Total assay volume was 0.2 ml. Reactants were incubated in a Multiscreen MHAF B3 H60 filter plates (as above) for 60 min at 25°C.

Subsequently, both assays were treated a similar manner: The reaction was terminated by rapid filtration of the multiscreen assay plates using a Millipore manifold at a pressure of 700 mbar. The filters were washed three times with 200 μl of the relevant assay buffer. Scintillation fluid (Perkin Elmer, Beaconsfield, UK) was added (100 μl/well), and bound radioactivity determined using a Wallac Microbeta scintillation counter (Beaconsfield, UK).

All results were expressed as a percentage of the mean CPMs obtained as a result of the receptor ligand reaction in the absence of any compound. Data was plotted using GraphPad Prism, Version 2.0 (GraphPad, San Diego, CA). The apparent K_i of the bound ligand was calculated for each active compound using the Cheng–Prusoff Equation. K_D values of 1.6 nM (human A_1 receptors) for [^{3}H]DPCPX and 1 nM (human A_{2A} receptors) for [^{3}H]ZM241385 were previously determined (data not shown).

A_{2B} Receptor Affinity

Due to the lack of a suitable radioligand the affinity of ligands at A_{2B} ARs was determined in adenylyl cyclase experiments [Klotz et al., 1998]. Concentration-dependent inhibition of NECA-stimulated adenylyl cyclase caused by antagonists was measured in membranes from CHO cells stably transfected with the human A_{2B} AR. Membranes were incubated with approximately 150,000 cpm of [α-^{32}P]ATP for 20 min and K_i-values were then calculated from the measured IC_{50}-values using the Cheng and Prusoff equation [Klotz et al., 1998].

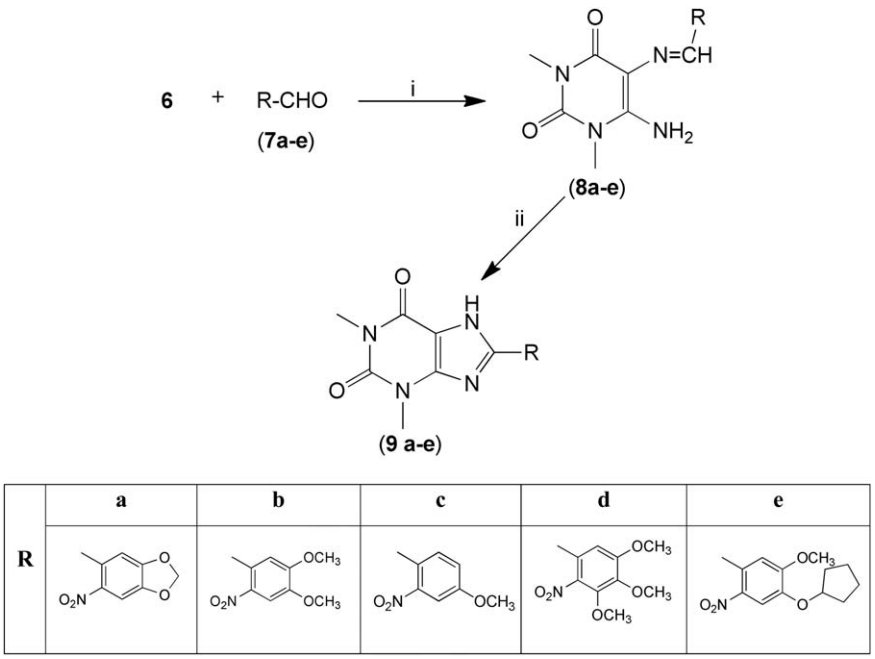


Fig. 3. Synthesis of 8-(2-nitroaryl)substituted xanthine derivatives **9a–e**. Reagents and reaction conditions: (i) MeOH/CH_3COOH, room temperature, 18 h; (ii) $SOCl_2$, reflux, 30–40 min; NH_4OH.

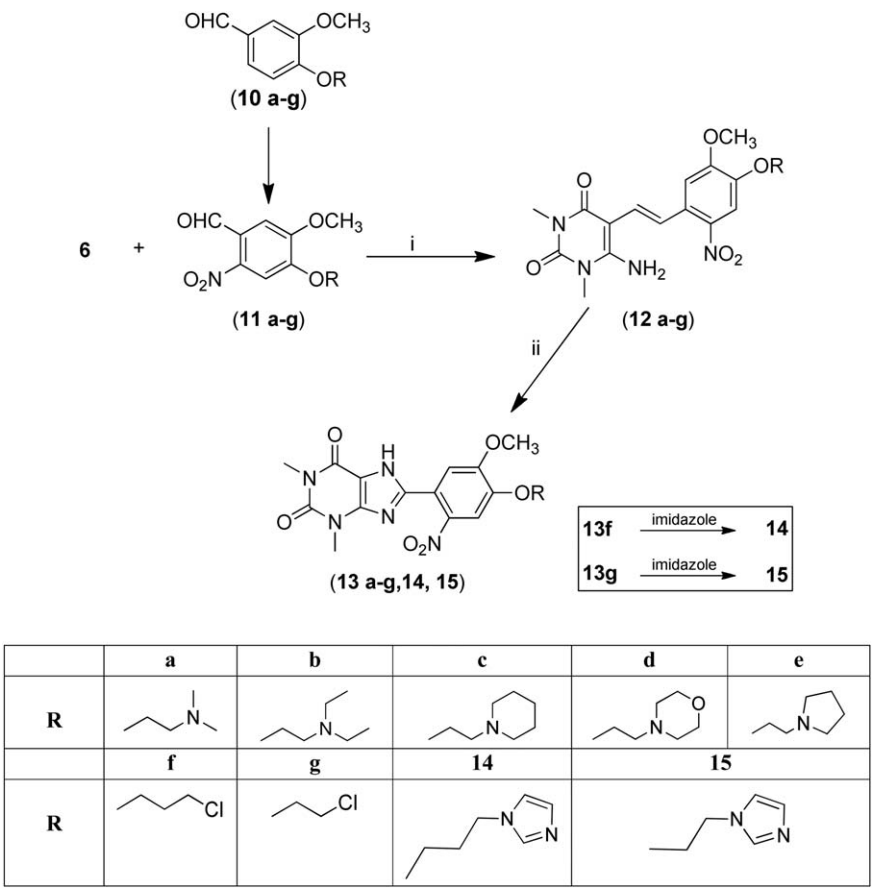
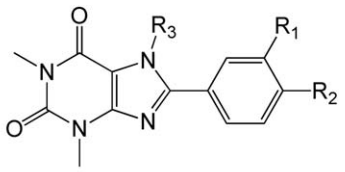


Fig. 4. Synthesis of 8-(2-nitroaryl)substituted xanthine derivatives **13a–g**, 14, 15. Reagents and reaction conditions: (i) MeOH/CH_3COOH, room temperature, 18 h; (ii) $SOCl_2$, reflux, 30–40 min; NH_4OH.



16-23

Comp No.	R_1	R_2	R_3
16	$-OCH_3$	$-OCH_2CH_2CH_2Cl$	$-CH_3$
17	$-OCH_3$	$-OCH_2CH_2CH_2Cl$	$-CH_2CH_2CH_3$
18	$-OCH_3$	$-OCH_2CH_2CH_2ONO_2$	$-CH_3$
19	$-OCH_3$	$-OCH_2CH_2CH_2ONO_2$	$-CH_2CH_2CH_3$
20	$-OCH_2CH_2CH_2Cl$	-H	$-CH_2CH_2CH_3$
21	$-OCH_2CH_2CH_2ONO_2$	-H	$-CH_2CH_2CH_3$

Fig. 5. Synthesis of 8-nitrooxypropoxyphenylxanthines **18**, **19**, and **21**. Reagents and reaction conditions: (i) $AgNO_3$, CH_3CN, 60°C, 18 h.

RESULTS AND DISCUSSION

Chemistry

The synthetic pathways for the synthesis of 8-(substituted phenyl)xanthines are depicted in Figures 2–5. 5,6-Diamino-1,3-dimethyluracil (**6**), the key intermediate for the synthesis of all 8-(substituted-phenyl)xanthines was synthesized according to reported methods [Papesch and Schroeder, 1951; Blicke and Godt, 1954] summarized in Figure 2.

Variously substituted nitroaldehydes (**7a–e**, **11a–g**) were treated with compound **6** to afford corresponding benzylidene derivatives **8a–e** and **12a–g**. A singlet integrating for one proton appeared at $\sim\delta$ 9.75 for N═C*H* in ^{1}H NMR spectra of all the benzylidenes. Subsequent cyclization [Senga et al., 1978] of these compounds using thionyl chloride yielded the desired corresponding 8-substituted 1,3-dimethylxanthines **9a–e** and **13a–g** (Figs. 3 and 4).

Imidazole derivatives **14** and **15** were also prepared by fusing **13f** and **13g** with imidazole at 120°C for 3 h [Bansal et al., 2011]. Compound structures were confirmed using various spectral analyses, which were consistent with the proposed structures as detailed under experimental section.

7-Alkyl-8-[4-(3-chloropropoxy]phenyl derivatives **16** and **17**, previously synthesized and described in earlier reports [Bansal et al., 2011], were treated with silver nitrate in acetonitrile at 60°C to obtain nitrate esters 18 and 19, respectively (Fig. 5). The structures of the compounds were confirmed using various spectral analyses. Asymmetric and symmetric stretching bands for ONO_2 were present near 1540 and 1280 cm^{-1}, respectively, in the IR spectra of both the compounds. A downfield shift (δ 4.72) of CH_2—ONO_2 protons was observed as compared to CH_2—Cl (δ 3.90) of precursor chloro derivatives **16** and **17** in the NMR spectra. To comprehend the effect of the substitution pattern of the phenyl ring, nitrooxyaryl derivative **21** of 7-alkyl-8-(*m*-substituted)-phenylxanthine (**20**) was also synthesized.

Adenosine Receptor Binding

The synthesized compounds were tested in binding assays using cloned human adenosine A_1 and A_{2A} receptors using [^{3}H]DPCPX and [^{3}H]ZM241385, respectively. Affinities of synthesized xanthine derivatives in radioligand binding assays at A_1 and A_{2A} receptors are summarized in Table 2.

The majority of the newly synthesized 8-(2-nitroaryl)xanthines displayed low affinity for ARs in comparison to their complementary nitro group unsubstituted xanthine derivatives [Bansal et al., 2009], however good selectivity for the A_{2A} subtype was observed with some compounds. 8-Nitroarylxanthine **9e** bearing a cyclopentyloxy moiety, showed 30 times higher binding affinity for adenosine A_{2A} receptors than A_1 and A_{2B} receptor subtypes. 8-(2-Nitrophenyl) xanthines substituted with pyrrolidinylethoxy (**13e**), chloroethoxy (**13g**) and imidazolylpropoxy (**14**) moieties were also selective for A_{2A} ARs. Introduction of nitro group at 2- position of 8-aryl ring of xanthines results in overall decreased affinity for ARs, however selection of a suitably substituted 8-nitrophenyl ring may result in improved selectivity for A_{2A} receptors a finding that may be useful for development of xanthine based A_{2A} selective ligands. Introduction of an

TABLE 2. AR Binding Affinities of Synthesized Xanthine Derivatives

Compound no.	A_1 K_i (μM)	A_{2A} K_i (μM)	A_{2B} K_i (μM)
(**9a**)	>30	5 (1.9–10)	>10
(**9b**)	>30	>30	>30
(**9c**)	>30	>30	>10
(**9d**)	>30	>30	>30
(**9e**)	>30	1 (0.69–1.6)	>30
(**13a**)	>30	>30	>30
(**13b**)	>30	>30	>30
(**13c**)	>30	>30	>30
(**13d**)	>30	>30	>30
(**13e**)	>30	4 (1.4–13)	>30
(**13f**)	>30	>30	>10
(**13g**)	>30	2 (1.5–2.0)	>30
(**14**)	>30	7 (0.75–14)	>30
(**15**)	>30	>30	>30
(**16**)	>30	0.7 (0.4–1.2)	–
(**17**)	>30	1 (0.6–1.5)	–
(**18**)	>30	4.8 (2.8–8.2)	>30
(**19**)	>30	0.68 (0.64–0.72)	>30
(**20**)	>100	2.9 (0.65–13)	–
(**21**)	>100	4.4 (3.83–5.12)	>30
DPCPX	0.095 (0.06–0.15)	0.13[a]	
ZM 241385	0.54[a]	0.064 (0.03–0.14)	

Shown is data from radioligand binding assays at A_1 and A_{2A} ARs and inhibition of NECA-stimulated adenylyl cyclase activity for the A_{2B} subtype.
Ki Values are given with 95% confidence limits.
[a]Reference [Klotz, 2000].

8-nitrooxyaryl moiety in compounds **18**,**19**, and **21** does not appear to have much effect on adenosine binding affinity in comparison to their chloropropoxy counterparts **16**, **17**, and **20**.

CONCLUSIONS

The newly synthesized 8-(2-nitroaryl)substituted xanthines displayed reduced binding affinity for ARs as compared to their corresponding structural analogues without a nitro moiety, however improved selectivity toward the A_{2A} receptor subtype was observed for some of the nitro phenyl substituted xanthines. Introduction of 8-nitrooxyaryl moiety does not appear to bring much change in adenosine binding affinity when compared with their chloropropoxy counterparts. The current study provides additional knowledge in the pursuit of substituted xanthines as selective A_{2A} AR ligands.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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