International Journal of Anatomy and Physiology ISSN: 2326-7275 Vol. 7 (9), pp. 001-005, September, 2018. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Pharmacological studies of some pyrimidino derivatives

Saleh A. Bahashwan

College of Health Sciences, Pharmacy Department, Taibah University, Medina Munawarah, Saudi Arabia.
E-mail: salehbahashwan@gmail.com. Tel: +966505308524. Fax: +9668450144.

Accepted 07 April, 2018

Pyrimidine derivatives, in general, have received significant interest due to their purported biological activities the anti-inflammatory, analgesic, anti-Parkinsonism, and anti-microbial activities for five 2[(N-methyl indolyl)-methyl] pyrimidino derivatives 1-5 is reported. Pharmacological screenings showed that these compounds have good anti-inflammatory, analgesic, anti-Parkinsonism, and anti-microbial activities comparable with reference drugs. Compound 2 showed similar activity of diclofenac sodium as analgesic. Compound 1, 2, 4 and flurbiprofen exhibited essentially equipotent anti-inflammatory activities. Compounds 1 and 3 were the most potent anti-Parkinsonism agents compared with benztropine. In addition, compounds 1-5 showed antimicrobial activities comparable to streptomycin, erythromycin and fusidic acid as reference drugs. These pharmacological studies provide the activities for new pyrimidino derivatives and are expected to possess notable chemical and pharmacological activities for further pharmacological research.

Key words: Pyrimidino, pharmacological, analgesic, anti-inflammatory, anti-Parkinsonism.

INTRODUCTION

Pyrimidines and fused pyrimidines, being an integral part of DNA and RNA, play an essential role in several biological processes and have considerable pharmacological importance, particularly, the pyrimidine ring can be found in nucleoside antibiotics, anti-bacterial, cardiovascular as well as agrochemical and veterinarian products (Fayed et al., 2009). Pyrimidines present an interesting group of compounds many of which possess wide-spread pharmacological properties such as anti-depressant, antimicrobial, anticancer, and anticon-vulsant activities (Hammam et al., 2003; Deeb et al., 2004; Abo-Ghalia and Amr, 2004; Amr, 2005; Amr et al., 2006a, b). Heterocyclic compounds have received considerable attention owing to their variety of biological activities, especially as inhibitors of PDE5 extracted from human platelets (Piaz et al., 2002), HIV-1 reverse transcriptase (Sweeney et al., 2008), human EPK2 (Takayoshi et al., 2006), cyclin-dependent kinas (Brana et al., 2005). Also, heterocyclic nitrogen compounds are indispensable structural units for medicinal chemists and used as antibiotics, anthelmintics, antiviral, anti-depressant, and anti-inflammatory (Ramaliagam et al., 2010; Shamroukh et al., 2007; Mansour et al., 2003). In view of these observations we evaluate some new pyrimidine derivatives 1-5 which were previously synthesized according to Ouf et al. (2008), for their analgesic, anti-inflammatory, anti-parkinsonism, and anti-microbial activities in comparison to some reference drugs (Figure 1).

MATERIALS AND METHODS

Male and or female albino mice weighing from 20 to 25 g were used and obtained from the Animal House Colony, Research Institute of Ophthalmology, Giza, Egypt. All animals were maintained according to standard international human care. The new compounds were screened pharmacologically for their analgesic, anti-inflammatory and anti-parkinsonism. Anti-microbial activities with different microorganism species were used.

Analgesic activity

Sixty albino mice were divide into 10 groups, one group was kept as control (received saline), the second group received vehicle (gum acacia), and the third received diclofenac sodium as a reference drug, where as the other groups received 1-5 compounds using a subcutaneous (S.C) administration. The mice were dropped

Table 1. Analgesic activity of compounds as compared with Diclofenac sodium in mice.

Compound no.	Analgesic activity after						
	10 min	20 min	30 min	45 min	60 min	90 min	120 min
Diclofenac sodium (ref.)	**1**	**1**	**1**	**1**	**1**	**1**	**1**
1	0.81	0.85	0.87	0.91	0.92	0.94	0.94
2	0.97	0.99	0.99	1.07	1.11	1.13	1.24
3	0.54	0.57	0.58	0.55	0.51	0.49	0.48
4	0.76	0.81	0.83	0.87	0.88	0.88	0.91
5	0.31	0.36	0.37	0.41	0.42	0.45	0.43

Table 2. Anti-inflammatory activity of compounds, (% reduction in edema induced by yeast).

Compound no.	Post treatment 3 h	Post treatment 6 h
Flurbiprofen (ref.)	**100 %**	**100 %**
1	25.8	36.6
2	-	-
3	28.2	37.1
4	7.4	16.9
5	22.5	31.2

gently in a dry glass beaker of 1 dm^3 capacity maintained at 55 to 55.5°C, normal reaction time in seconds for all animals were determined at time interval of 10, 20, 30, 45, 60, 90 and 120 min. The reaction time is defined as time elapsed from the mouse reaches the hot beaker till the animal licks its feet or jump out of the beaker (dose 5 mg/kg) (Tgølsen et al., 1991). Relative potencies to diclofenac sodium were also determined (Table 1).

Anti-inflammatory activity

Five compounds 1-5 were evaluated for their anti-inflammatory activity. Ninety-five mice, divided into nineteen equal groups were used. Edema in the mice paw was induced by injection of 0.1 ml of 20% Brewer's yeast suspended in physiological saline solution in the paw skin of the hind limb. After 4 h, the thickness of the paw was measured using a skin caliber to detect the inflammation induced by the yeast (Van Dyke et al., 1982). The first group was left as control while the second group was injected intraperitonialy (I. P) with dimethyl sulfoxide (DMSO) and the third group was injected (I. P) with flurbiprofen (20 mg/kg). The remaining groups were treated with the tested compounds dissolved in DMSO in a dose of 100 mg/kg (I.P). The paw thickness was measured after 5 and 6 h, post injection (Table 2).

Anti-Parkinsonism activity

The muscarinic agonists' tremorine and oxotremorine induced Parkinsonism like signs such as tremor, ataxia, spasticity, salivation, lacrimation, and hypothermia. These signs are anta-gonized by anti-parkinsonism agents. Groups of eight male albino mice (12 to 20 g) were used. They were dosed orally with the tested compounds (5 mg/kg) or the standard (Benztropine mesylate, 5 mg/kg) (Abdel-Latif et al., 2007) 1 h prior to the administration of 0.5 mg/kg of oxotremorine. Rectal temperature was measured before administra-tion of the compounds and 1 h after oxotremorine dosage. The scores for the recorded signs are zero (absent), one (slight), two (medium), and three (high)(Table 3).

Anti-microbial activity with microorganism species

(1) Bacteria

(a) Gram- negative bacteria*: Escherichia coli*, *Salmonella typhimurium*.
(b) Gram-positive bacteria: *Bacillus subtilis, Staphylococcus aureus, Streptococcus mutans.*
(2) Fungi: *Aspergillus niger*, *Candida albicans.*
(3) Yeast*: Sacchromyces cerevisiae*.

Medium

The cap- assay method (Bauer et al., 1966) containing: peptone (6 g/L), yeast extract (3 g/L) meat extract (1.5 g/L), glucose (1 g/L) and agar (20g/L) were used. The medium was sterilized and divided while hot (50 to 60°C) in 15 ml, portions among sterile Petri -dishes of 9 cm diameter. 1 ml of the spur suspension of each micro-organism was spread all over the surface of the cold solid medium placed in the Petri-dish.

Method

0.5 g of each of the tested compounds was dissolved in 5 ml of dimethyl formamide. An amount of 0.1 ml of test solution was placed on Whatman paper disc of 9 mm diameter and the solvent was left to evaporate. These saturated discs were placed carefully on the surface of the inoculated solid medium; each Petri- dish contains at least 3 discs. The Petri- dishes were incubated at 5°C for an hour to permit good diffusion and then transferred to an incubator of 85°C overnight then examined. The results were recorded by measuring the inhibition zone diameters (Table 4).

Table 3. Anti-Parkinsonism activity of several compounds as compared with benztropine.

Compound no.	Salivation and lacrimation	Tremors score	% decrease from oxotremerine rectal temperature	Relative potency to Benztropine
Benztropine (control)	1	1	26	1
1	1	1	19	0.81
2	2	2	11	0.45
3	1	1	20	0.82
4	2	2	16	0.61
5	2	2	15	0.42

Table 4. Antimicrobial activities of the tested compounds.

Tested compound and positive drug	Inhibition zone (mm)							
	Micro-organism							
	Bacteria					Fungi		Yeast
	Gram +ve		Gram –ve					
	E. coli	*S. typhimurium*	*B. subtilis*	*S. aureus*	*S. mutans*	*A. niger*	*C. albicans*	*S. cerevisiae*
Control DMSO/DMF	-	-	-	-	-	-	-	-
Streptomycin	+++	+++	+++	+++	+++	+	+++	+++
Erythromycin	+	-	+++	++	++	-	-	-
Fucidic acid	-	-	-	-	-	+++	+++	+++
1	+++	++	+++	+++	++	+	+++	+++
2	+++	+++	+++	++	++	+	+++	++
3	-	++	+	-	-	-	+++	-
4	-	-	+	-	++	+	+	+
5	+	++	-	+	-	+	++	++

+++High sensitive (inhibition zone = 21-40 mm), ++ Fairly sensitive (inhibition zone =16-20mm), +Slightly sensitive (inhibition zone =10-15 mm). - No sensitive

RESULTS AND DISCUSSION

Four pharmacological activities namely; analgesic, anti-inflammatory, anti-parkinsonism, and anti-microbial activities were tested despite their different biological receptors. Five representative compounds 1- 5 (Figure 1) were studied with respect to their analgesic, anti-inflammatory, anti-parkinsonism, and anti-microbial activities. The activities of these compounds are different according to the structure and function groups (Tables 1 to 4).

Analgesic activity

All tested compounds exhibited analgesic activities (Table 1), the most potent is 2 that showed the same activity as diclofenac sodium after 45 min and it had even higher activity than

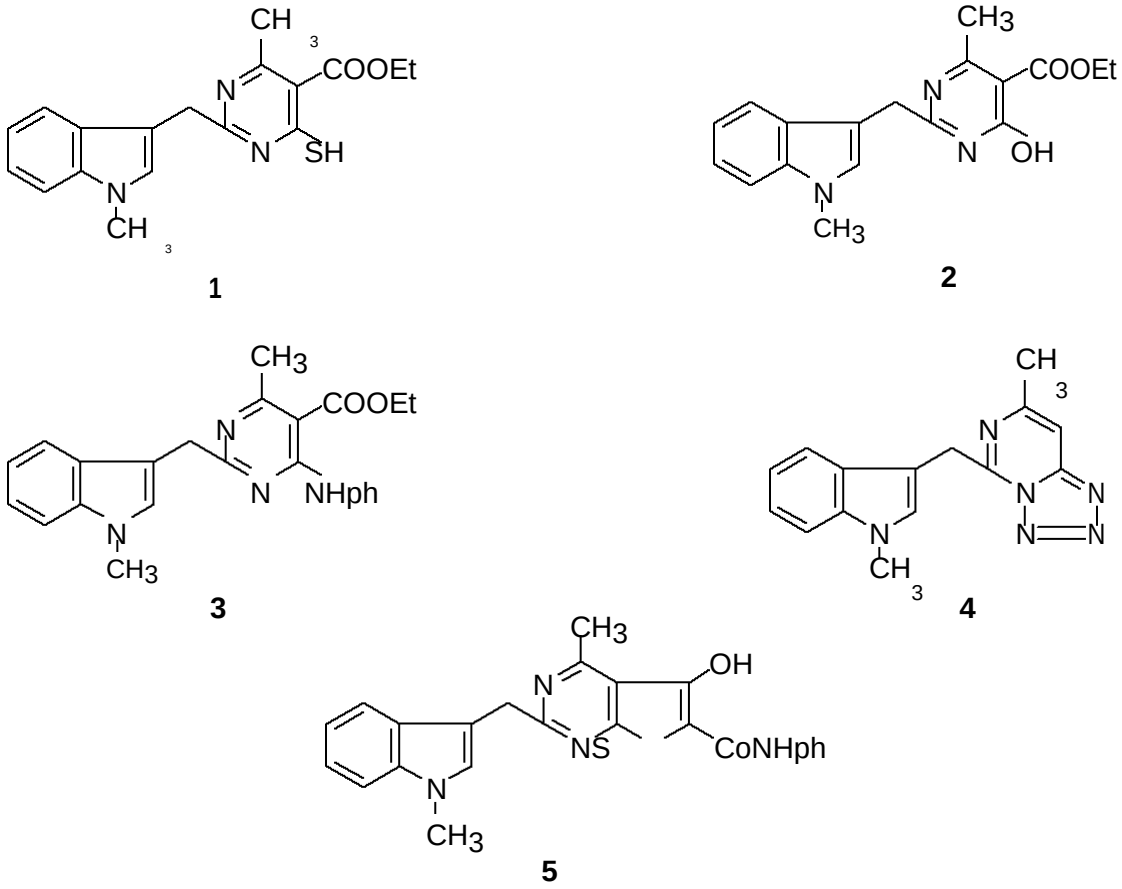

Figure 1. Pyrimidine derivatives 1-5.

diclofenac sodium after 60, 90 and 120 min. Also the analgesic activities of 1 and 4 approached those of diclofenac sodium, and 5 had 31 to 45% activity (Table 1).

Anti-inflammatory activity

From Table 2, it is appeared that compounds 1- 5 have significant anti-inflammatory activities. Here, their lipophilic property seems to play an important role. Where, the most active compounds 1, 3, 4 and the standard drug flurbiprofen were found to exhibit essentially equipotent anti- inflammatory activity. Compound 4 was found to lower anti- inflammatory activities, while compound 2 showed no significant anti-inflammatory activities.

Anti-parkinsonism activity

Compounds 2, 4 and 5 showed moderate activity (relative potencies to benztropine 0.44, 0.60 and 0.40). Compounds 1 and 3 are the most potent anti-Parkinsonism agents (0.80 relative potency) (Table 3).

Anti-microbial activity

The organic compounds 1-5 were evaluated for its anti-microbial activity against five bacterial strains *Escherichia coli*, *Salmonella typhimurium, Bacillus subtilis, Staphylococcus aureus* and *Streptococcus mutans* and two fungal strains *Aspergillus niger* and *Candida albicans* and also one strain of yeast *Saccharomyces cerevisiae* at 50 µg/ml concentration according to modified Kirby-Bauer's disk diffusion method. Minimum inhibition concentration (MIC) values of tested compounds were determined by tube dilution technique. The solvent DMSO/DMF was used as negative controls while streptomycin, erythromycin and fusidic acid was used as standards. Calculated average diameters (for triplicate sets) of the zones of inhibition (in mm) for test samples were compared with that produced by the standard drugs.

Almost, all tested compounds were found to exhibit anti-microbial activities. Analysis of anti-microbial data suggested that compounds 1 and 2 possessed higher significant anti-bacterial and anti-fungal activities than some known standard drugs. The results of anti-microbial screening were recorded as average diameter of inhibition zone in mm and summarized in Table 4.

Conclusion

Pyrimidino derivatives 1-5 have been noted to possess pharmacological screening as analgesic, anti-inflammatory, anti-Parkinsonism and anti- microbial activities comparable to reference drugs. All tested compounds exhibited analgesic activities and compound 2 showed the same activity as diclofenac sodium after 45 min. and exhibited higher activity after 60, 90 and 120 min. The most active compounds 1, 3, 4 and standard drug flurbiprofen were found to exhibit essentially equipotent anti-inflammatory activity. Also, compound 1 and 3 exhibited anti-Parkinsonism even higher activity of benztropine as standard drug. All compounds tested were found to exhibit anti-microbial activities, where compound 1 and 2 possessed higher significant antibacterial and antifungal activities than some known standard drugs as streptomycin, erythromycin and fusidic acid .

ACKNOWLEDGEMENT

The kind help of Dr. Ahmed A.S. Fayed, National Research Center, Applied organic chemistry department, Cairo, Dokki, Egypt, for carrying out the synthesis chemical compounds are acknowledged.

REFERENCES

Abdel-Latif NA, Sabry NM, Mohamed AM, Abdulla MM (2007). Synthesis, Analgesic and Anti-Parkinsonism profiles of some pyridine pyrazoline and Thinopyrimidine derivatives. Monatsh. Chem., 138: 715 – 724.

Abo-Ghalia MH, Amr AE (2004). Synthesis and investigation of a new cyclo-(N -dipicolinoyl) pentapeptide of a breast and CNS cytotoxic activity and an ionophoric specificity. Amino Acids, 26: 283-289.

Amr AE, Abdel -Latif NA, Abdalla MM (2006a). Synthesis of some new testosterone derivatives fused with substituted pyrazoline ring as 5 - redutase inhibitors. Acta. Pharm., 56: 203-218.

Amr AE, Abdel-Latif NA, Abdalla MM (2006b). Synthesis and Androgenic activity of some new 3-substituted androstano [17, 16-c]-5-aryl pyrazoline and their derivatives. Bioorg. Med. Chem., 14: 373-384.

Amr AE, Sayed HH, Abdalla MM (2005). Synthesis and reaction of some new substituted pyridine and pyrimidine derivatives as analgesic, anti-convulsant and anti-parkinsonian agents. Arch. Pharm. Chem. Life Sci., 338: 433-440.

Amr AE (2005). Synthesis of some new linear and chiral macrocyclic pyridine carbazides. Z Naturforsch, 60b: 990-998.

Bauer AW, Kirby WM, Sherris JC, Turck M (1966). Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Path., 45: 493–496.

Brana MF, Cacho M, Garcia ML, Mayoral EP, Lopez B, de Pascual-Teresa B (2005). Pyrazolo [3, 4-c] pyridazines as novel and selective inhibitors of cyclin dependent kinases. J. Med. Chem., 48(22): 6843–6854.

Deeb A, El-Mariah F, Hosny M (2004). Synthesis and antimicrobial activity of some pyridazino[3,4:3,4] pyrazolo[5,1-c]-1,2,4- triazines. Bioorg. Med. Chem. Lett., 14: 5013 - 5017.

Fayed AA, Hosni HM, Flefel EM, Amr AE (2009). Synthesis and pharmacological activities of some new thieno [2,3-d] pyrimidine and pyrimidino pyrazolo thieno pyrimidine derivatives. W. J. Chem., 4: 58-65.

Hammam AG, Fahmy AFM, Amr AE, Mohamed AM (2003). Synthesis of novel tri-cyclic heterocyclic compounds as potential anti-cancer agents using chromanone and thiochromanone as synthons. Ind. J. Chem., 42: 1985-1993.

Mansour AK, Eid MM, Khalil NSAM (2003). Synthesis and reactions of some new heterocyclic carbohydrazides and related compounds are potential anti-cancer agents. Molecule, 8: 744 – 755.

Ouf NH, Amr AE, Fayed AA (2008). Synthesis, reaction, and pharmacological activities of some pyrimidines using (N-methyl indolyl) acetic acid as synthon. Monatsh. Chem., 139: 281 – 287.

Piaz VD, Castellana MC, Vergelli C, Giovannoni MP, Gavalda A, Segarra V, Beleta J (2002). Synthesis and evaluation of some pyrazolo [3, 4-d] pyridazinones and analogues as PDE 5 inhibitors potentially useful as peripheral vasodilator agents. J. Enzyme Inhibition Med. Chem., 17: 227 -233.

Ramalingam P, Ganapaty S, Babu Rao Ch (2010). In vitro anti-tubercular and anti-microbial activities of 1-substituted quinoxaline-2, 3(1H, 2H) diones. Bioorg. Med. Chem. Lett., 20: 406–408.

Shamroukh AH, Zaki MEA, Morsy EMH, Abdel-Motti FM, Abdel-Megeid FME (2007). Synthesis of pyrazolo [4, 3:5, 6] pyrano [2, 3-d] pyrimidine derivatives for anti-viral evaluation. Arch. Pharm., 340: 236–243.

Sweeney ZK, Harris SF, Arora NN, Javanbakht HH, Li YY, Fretland JJ (2008). Design of annulated pyrazoles as inhibitors of HIV-1 reverse transcriptase. J. Med. Chem., 51(23): 7449–7458.

Takayoshi K, Masaichi W, Makoto O, Kentaro S, Masahiro N, Takashi F (2006). Crystal structure of human ERK2 complexed with a pyrazolo [3, 4-c] pyridazine derivatives. Bioorg. Med. Chem. Lett., 16: 55 58.

Tjølsen A, Rofland G H, Berge Og, Hole K (1991). The increasing temperature hot-plate test: An improved test of nociception in mice and rats. J. Pharmacol. Meth., 25: 241–250.

Van Dyke K, Peden D, VanDyke C, Jones G, Castranova V, Ma, J (1982).Inhibition by non-steroidal anti-inflammatory drugs of iminol dependent human- granulocyte chemiluminescence and [3H] FMLP binding. Inflammation, 6: 113–125.