Original Research Article

Anti-parkinsonian, anti-inflammatory, anti-microbial, analgesic, anti-hyperglycemic and anticancer activities of poly-fused ring pyrimidine derivatives

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Abstract

Purpose: To investigate the anti-inflammatory, anti-cancer, anti-parkinsonian, anti-microbial, analgesic, and anti-hyperglycemic properties of a variety of poly-fused pyrimidine derivatives.
Methods: A novel series of fused pyrimidine derivatives 1-6 were synthesized by reacting amino pyrazole derivatives with active methylene derivatives to give the corresponding compounds 1-6. Anti-parkinsonian activity was investigated with benzotropene as a reference drug, anti-inflammatory effect in mice was evaluated using carrageenan in paw edema with indomethacin as reference drug, anti-microbial activity was assessed using nutritional agar with ciprofloxacin and ketoconazole as reference drugs, analgesic activity was evaluated using valdecoxib as reference drug, anti-hyperglycemic activity was investigated using alloxan and sucrose models (SLM) with pioglitazone as reference drug and anticancer activity was investigated using the MTT micro-cultured tetrazolium assay method with doxorubicin as reference drug.
Results: Pyrimidine derivatives 1-6 possess significant active anti-parkinsonian, anti-inflammatory, anti-microbial, analgesic, anti-hyperglycemic and anticancer activities in comparison to the reference drugs used for each model. Compared to Benzotropene®, compounds 1 and 3 showed a significantly stronger anti-parkinsonian activity ($p < 0.03$). Compound 3 showed a significantly stronger anti-inflammatory effect than Indomethacin® ($p < 0.05$). Compounds 5 and 6 exhibited significant anti-microbial activity compared to ciprofloxacin® ($p < 0.05$). Compounds 4 and 6 exhibited significantly improved analgesic activity as compared to Valdecoxib® ($p < 0.01$). Compounds 1 and 3 exhibited significantly higher anti-hyperglycemic effects in SLM model when compared to pioglitazone® ($p < 0.02$). Compound 5 demonstrated the highest activity against human colon cancer cell line (HT-29) and human prostate cancer cell line (DU145), and also, significantly improved level of efficacy against human lung cancer cell line (A549) compared to Doxorubicin® ($p < 0.02$).
Conclusion: Using the six pyrimidine derivatives 1 - 6 as a lead molecule, a novel class of clinically beneficial anti-cancer, anti-inflammatory, anti-microbial, analgesic, and anti-hyperglycemic drugs may be produced.

Keywords: Pharmacological, Pyrimidine, Anti-Parkinsonian, Anti-inflammatory, Analgesic, Anti-hyperglycemic, Anticancer

INTRODUCTION

Pyrimidine derivatives are especially of great value in pharmacology and medicine. Pyrimidine compounds have been studied for their potential antiangiogenic effects, kinase and cGMP phosphodiesterase inhibition [1]. It has been discovered that pyrimidines are useful in the treatment of diabetes [2], effective against *Mycobacterium tuberculosis*, and potent antifungal, and antibacterial agents [3]. Pyrimidines possess properties such as anti-inflammatory, analgesic [4], herbicidal, antallergic, antihistaminic [5], anticonvulsant and antidepressant, and free of neurotoxicity [6], anticancer, and antioxidant properties [7]. Parkinson's disease (PD) is a neurological condition affecting 1 – 2 % of persons over 65, typified by the selective death of dopaminergic neurons in the substantia nigra (SN) [8]. Diabetes mellitus, a metabolic disease characterized by inadequate insulin synthesis from pancreatic cells, is one of the three main causes of death globally [1]. Hyperglycemia caused by abnormalities in the manufacture or action of insulin, or both, are used to identify it. Lipid marker alterations are linked to hyperglycemia and may have a role in cardiovascular problems. Due to the rise in multi-drug resistance, microbial pathogens and the emergence of new infectious diseases, treatment of infectious diseases remains a major and difficult problem. Even with an array of antibiotics and chemotherapeutics, the emergence of antibiotic-resistant bacterial strains necessitates the development of new classes of powerful antimicrobial drugs. Heterocyclic compounds have played a major role in the development of a new class of structural entities with unique modes of action that have important therapeutic applications. There is ample evidence supporting the antibacterial, analgesic, anti-inflammatory, anticancer, anticonvulsant, and antimalarial characteristics of these heterocyclic compounds [9]. Based on these results, and previously published studies, a few new poly heterocyclic fused ring systems with pyrazole-pyrimidine moiety were synthesized and investigated for various pharmacological activity.

EXPERIMENTAL

Pharmacological screening

In collaboration with King Saudi University (Riyadh, KSA), Taibah University's animal home in Madinah Munawara, KSA, male and female Swiss albino mice weighing 16 – 18 g were provided. The College of Pharmacy at Taibah University's Research Ethics Committee approved the study (approval no. COPTU-REC-50-20230119). Treatment of the animals complied with accepted guidelines for animal care [10].

Anti-parkinsonian activity

The male Swiss albino mice were randomly assigned into 8 groups. Oral administration of either the standard (5 mg/kg) or the tested compounds (5 mg/kg) benzotropene mesylate was administered [11]. An hour later, oxotremorine® (0.5 mg/kg) was administered subcutaneously (SC). Before and after the oxotremorine® injection, the rectal temperature was taken. The following scores were assigned to the signs that were observed: zero for none, one for slight, two for medium, and three for high.

Anti-inflammatory activity

The anti-inflammatory activity was investigated using the carrageenan-induced paw edema method. Compounds 1 to 6 were dissolved in a uniform 0.5 % carboxymethyl cellulose (CMC) solution. A total of one hundred and eight mice were randomly assigned to eighteen groups, each consisting of six animals. The reference drug, indomethacin ®, and the tested chemical substance were administered orally in an aqueous suspension at a dose of 5 mg/kg. Animals used as controls only got 0.5 % CMC. A sample of 0.01 mL of freshly prepared 1.0 % carrageenan solution (in formol saline) was injected into the sub-plantar region of the right hind paw. A digital plethysmometer (Model 7150) was used to measure the volume of the right hind paw before and after administration of the investigated drugs at 1, 2, and 3 h intervals [12].

Ulcerogenic activity

The ulcerogenic activity of the tested substances and indomethacin® were assessed at 10, 50, and 100 mg/kg. Control mice were given 0.5 % CMC for 24 h before being given the tested compounds. After 6 h, the animals were sacrificed, the stomach was removed and opened along the major curvature, washed with distilled water, and gently cleansed with saline. Mucosa damage in each stomach was examined using a stereoscopic microscope compared to Indomethacin® [13].

Acute toxicity

The median lethal doses (LD50) of the tested compounds 1-6 were also investigated [13]. Adult male mice were injected intraperitoneally (i.p.) with progressive doses of each of the test

chemicals in groups of six. Percentage death was calculated for each animal group 24 h following injection. The LD_{50} was calculated graphically.

Anti-microbial activity

Using a nutritional agar medium, the novel synthesized compounds (1-6) were evaluated against four bacterial strains (*Staphylococcus aureus, Escherichia. coli, Klebsiella pneumoniae,* and *Bacillus subtilis*), and their antifungal activity was tested against two fungal strains (*Candida albicans* and *Aspergillus fumigates*) using sabouraud dextrose agar media. Six compounds (1-6) were tested for antibacterial activity *in vitro* using the agar diffusion method [14]. At 45 °C, the suspension of organisms was transferred into a sterile nutrient agar medium, which was then transferred to a sterile petri plate and allowed to harden. A cork borer was used to make holes (10 mm) in diameter, and filled with the solution of synthesized compounds (100 μg/mL). As a control, a dimethyl sulphoxide (DMSO-filled) hole was used. During pre-incubation time, the plates were placed at room temperature for 1 h and incubated for 24 h at 37 °C, and tested for antibacterial activity. Inhibition zone diameters were measured and compared to the standards (Ciprofloxacin® at 50 g/mL and Ketoconazole® at 50 g/mL).

The minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) of the compounds was assessed using the agar streak dilution method. The compounds under examination were produced as stock solutions in DMSO (68 mg/mL) as solvent. This stock solution was used to create a range of concentrations (0.17, 0.34, 0.68, 1.3, and 2.6 mg/mL), which were then aseptically mixed with melted sterile agar medium. Thereafter, 20 mL of the medium was added to a sterile petri dish and allowed to solidify. Next, one by one, microorganisms were streaked aseptically onto agar plates. Following streaking, all plates were incubated for 24 - 48 h at 37°C to test for antifungal and antibacterial properties [15]. The lowest concentration of the synthesized compound that inhibited the growth of bacterium/fungus was considered the minimum inhibitory concentration (MIC).

Analgesic activity

A total of 60 mice were randomly assigned to 6 groups. While the other groups received the test drugs (1–6) subcutaneously (SC), one group was given formol saline as a control, and gum acacia

as a carrier. After that, the mice were carefully placed into a one-liter dry glass beaker at 55 to 55.5 °C. All animals were given the drugs at 5 mg/kg/body, and after that, typical reaction times in seconds were measured at 10, 30, 60, and 120 min intervals. This is the period that elapses from the moment the mouse enters the heated beaker until it either jumps out or licks its feet [16].

Anti-hyperglycemic activity

A total of ten (10) groups comprising six male albino mice (16 – 18 g) were randomly assigned to control and test groups. The reference standard drug Pioglitazone® was administered intraperitoneally (i.p) at 0.6 g/kg. There were two phases of the animal study. Phase 1 assessed the capacity of tested drugs (1–6) to lower blood glucose in mice using the gavage tube and alloxan model (0.6 gm/kg body weight). Phase 2 assessed blood glucose using the sucrose mode model (SLM) (0.6 gm/kg body weight) [17].

Anticancer activity

Test compounds (1–6) were screened for anticancer activity on human colon cancer cell line (HT-29), human lung cancer cell line (A549), and human prostate cancer cell line (DU145) using the Montessori Teacher Training (MTT) micro-cultured tetrazolium assay method [18] and doxorubicin as reference drug. Logarithmic phase cultures were used to collect cells, which were then re-suspended in Dulbecco's modified Eagle's media (DMEM) with 10 % fetal bovine serum (FBS). After adjusting the cell counts, the same amount of cells were plated into each well of a 96-well culture plate, and the cells were left to grow for 12 h at 37 °C with 5 % CO_2. Test drugs were applied to the cells for 72 h. Thereafter 0.5 % DMSO saturated with CO_2 was added to the vehicle control culture's wells. Every 24 h, new culture media enriched with test chemicals was added. The microplate was then incubated for 4 h at 37 °C with 5 % CO_2 after 0.5 mg/mL of MTT reagent was added. Finally, the solute solution was added to thawed cells, and they were left to incubate for 12 h at 37 °C. Absorbance was measured in a microplate reader (Bio-Rad, USA) at 540 nm following the formazan crystals' full dissolution. For the determination of IC_{50} for the test substances, quadruple well results were analyzed.

Statistical analysis

Results were presented as mean ± standard error of the mean (SEM). Sigma Plot software (SPSS Inc., Chicago, USA) was used for

statistical analyses. Significant differences were determined using one-way analysis of variance (ANOVA) and Tukey's post-hoc test. $P < 0.05$ was considered statistically significant.

RESULT

Chemistry

A series of pyrimidine derivatives 1-6 were synthesized from 6-(naphtha(1,2-d)(1,3)thiazol-2-yl)-1,6-dihydro-pyrazole (3,4-c) pyrazol-3-amine according to established methods [19] (Figure 1, Table 1).
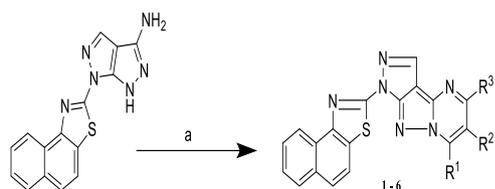
Scheme 1 : Reagent and condition : acetyl acetone, ethyl acetoacetate, ethyl cyanoacetate, diethyl malonate or malononitrile
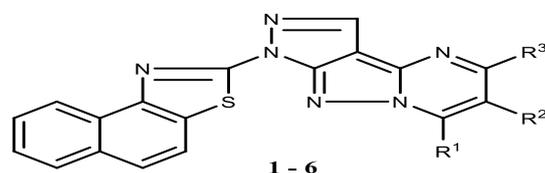

Figure 1: Pyrimidine derivatives **1-6**

Table 1: Substitutions on parent ring

Compound	R^1	R^2	R^3
1	OH	H	CH_3
2	CH_3	H	CH_3
3	NH_2	H	NH_2
4	NH_2	COPh	Ph
5	NH_2	CN	Ph
6	NH_2	COOEt	Ph

Pharmacological properties

Anti-parkinsonian, anti-inflammatory, ulcerogenic, anti-microbial, analgesic, anti-hyperglycemic, and anticancer were the seven pharmacological activities that were examined.

Tests were conducted on six representative compounds (1-6). These compounds' activity varied depending on their functional groups and structural makeup.

Anti-parkinsonism activity

When compared to Benzotropene® (1.00 ± 0.02), the anti-parkinsonian results revealed that compounds **1** and **3** had strong anti-parkinsonian activity (0.76 ± 0.01, 0.82 ± 0.02 of inhibition), compounds **2**, **4** and **6** had intermediate anti-parkinsonian activity (0.65 ± 0.02, 0.58 ± 0.03, 0.51 ± 0.02 of inhibition), and compound 5 had weak anti-parkinsonian activity (0.31 ± 0.01 of inhibition) (Table 1).

Anti-inflammatory activity

The results of the anti-inflammatory activity after 1 h showed that compound 5 has poor anti-inflammatory action (10.6 ± 1.3), while compounds 1, 2, 4, and 6 have intermediate activity (30.3 ± 1.2, 27.2 ± 1.3, 22.3 ± 1.4, and 21.4 ± 1.1, respectively) after 1 h. Compound 3 (51.6 ± 1.2) exhibited comparable activity with the reference (Indomethacin®) (57.4 ± 1.3). Similar results were obtained after 2 h and 3 h. Therefore, compound 3 possesses comparable anti-inflammatory activity (62.3 ± 1.1) with the reference drug Indomethacin® (65.4 ± 1.2) (Figure 2).
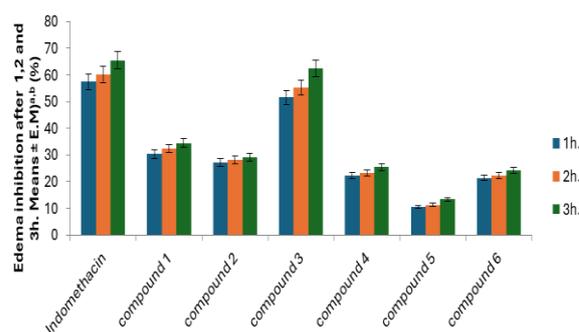

Figure 2: Anti-inflammatory activity of tested compounds 1-6. $P < 0.05$ *vs. indomethacin*

Table 1: Anti-parkinsonian activity of tested compounds **1-6**

Compound	Salivation and lacrimation Score	Tremors Score	Decrease from oxotremerine® (%)	Relative potency to Benzotropene®
1	3	2	21	0.76±0.01
2	2	2	15	0.65±0.02
3	3	3	24	0.82±0.02
4	2	2	16	0.58±0.03
5	1	1	10	0.31±0.01
6	2	1	13	0.51±0.02
Benzotropene®	1	1	26	1.00±0.02

Note: N = 6 rats, *$P \leq 0.05$ vs. control

Table 2: Gastric ulceration in mice

Compound	Dose (mg/kg)		
	10	**50**	**100**
Control	0/6[a]	0/6[a]	0/6[a]
Compound 3	0/6(0)	0/6(0)	0/6(0)
Indomethacin®	3/6(1.2±0.2)[b,c]	5/6(1.6±0.2)[b,c]	6/6(1.9±0.2)[b,c]

Note: a: Number of mice lesions bigger than 0.5 mm in length per total no of mice. b: SEM denoted the standard error of the mean, Number of animals N = 6 rats. c: *P* < 0.05 vs. control

Ulcerogenicity

Result for the ulcerogenic activity of compound 3 is presented in Table 2. Compound 3 showed no significant ulcerogenic activity in comparison to the control group.

Acute toxicity

Results for the determination of median lethal doses (LD$_{50}$) for compounds 1, 2, 3, 4,5 and 6 are presented in Table 3.

Table 3: Acute toxicity (LD$_{50}$) of selected compounds

Compound	LD$_{50}$ (g/kg)
1	2.54±0.012
2	2.11±0.013
3	2.07±0.012
4	1.32±0.012
5	2.29±0.013
6	2.31±0.011
Indomethacin®	1.81±0.015

Anti-microbial activity

Compound 1 significantly inhibited *S. aureus*, *E. coli*, *K. pneumoniae*, *B. subtilis*, and *C. albicans* (*p* < 0.05). Compound 2 significantly inhibited *S. aureus* and *E. coli* (*p* < 0.05). Compound 3 did not inhibit *A. fumigates*, *B. subtilis*, or *K. pneumoniae*, but it inhibited *S. aureus*, *E. coli* and *Candida albicans*. Compound 4 showed relatively poor inhibition against *C. albicans* and

A. fumigates but significant inhibition against *S. aureus*, *E. coli*, *K. pneumoniae*, and *B. subtilis* (*p* < 0.05). Compounds 5 and 6 significantly inhibited *S. aureus*, *E. coli*, *K. pneumoniae* and *B. subtilis*, as well as *C. albicans* and *A. fumigates* when compared to ciprofloxacin and ketoconazole respectively (*p* < 0.05) (Table 4).

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC, g/ ml) of the two most active compounds (5 and 6) against two bacteria (*S. aureus* and *B. subtilis*) and one fungus (*C. albicans*) was determined (Table 5). Compounds 5 and 6 significantly inhibited the selected bacterial and fungal strains in comparison with control (*p* < 0.05).

Analgesic activity

Compounds 3 and 5 showed minimal analgesic activity (0.31 ± 0.01, 0.41 ± 0.03 respectively) compared to Valdecoxib® (1.00 ± 0.01). It was shown that compounds 4 and 6 had high activity (0.72 ± 0.03, 0.78 ± 0.02 respectively), but compounds 1 and 2 had intermediate activity (0.64 ± 0.01, 0.53 ± 0.02 respectively). Compound 4 exhibited significantly higher analgesic activity (0.91 ± 0.08) after 30 mins (*p* < 0.05). Compound 4 had significantly greater action (1.12 ± 0.01) than Valdecoxib® (1.00 ± 0.01) after 60 mins.

Table 4: Anti-microbial activity of tested compounds at µg/mL concentrations

Compound	Diameter of inhibition Zone (mm)[a] (MIC values are in µg/mL)					
	Microorganism*					
	Antibacterial				Antifungal	
	S. aureus	*E. coli*	*K. pneumoniae*	*B. subtilis*	*C. albicans*	*A. fumigatus*
1	14	12	11	11	13	9
2	16	17	12	10	11	10
3	12	14	13	11	9	15
4	9	11	10	8	11	12
5	19	20	21	19	18	19
6	20	19	19	18	21	20
DMSO (solvent)	0	0	0	0	0	0
Ciprofloxacin[b]	22	21	23	20	-	-
Ketoconazole[b]	-	-	-	-	22	21

Note: [a]15 mm or less: resistant or no inhibition, 16 – 20 mm: moderate inhibition, 20 mm or more: maximum inhibition, [b]The concentration of the used standard drug was 50 µg/mL, *p* < 0.05 vs. control

Compounds 4 and 6 showed significantly higher activity (1.27 ± 0.04, 1.23 ± 0.04), respectively, while compound 1 showed high activity (1.02 ± 0.03) compared to Valdecoxib® (Figure 3).

Table 5: The minimum inhibitory concentration (MIC, µg/ml) of tested compounds 5 and 6

Selected organisms	Minimum inhibitory concentration (MIC)		
	5	*6*	*Standard[a]*
S. aureus	0.51	0.48	0.46
B. subtilis	0.45	0.52	0.38
C. Albicans	0.23	0.12	0.17

Note: [a]Ciproflaxacin and ketoconazole were used as standard drugs against bacterial and fungal strains, respectively
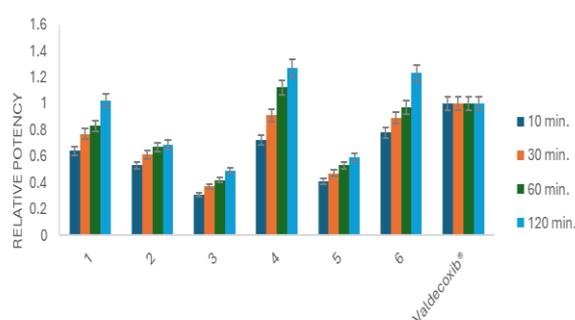

Figure 3: Analgesic activity of tested compounds 1 - 6. *P* < 0.05 vs. valdecoxib

Hypoglycemic activity in alloxan-induced diabetic rats

All investigated drugs demonstrated hypoglycemic effect in alloxan-induced diabetic rats. Compounds 1 and 3 had significantly high hypoglycemic effect (80.7 ± 2.2, 82.2 ± 2.4 mg/dL, respectively) after 24 h (*p* < 0.05). Also, compounds 1 and 3 have significantly high hypoglycemic effect (81.2 ± 2.3, 84.4 ± 2.4 mg/dL) after 72 h and 120 h (82.6 ± 2.5, 87.5 ± 2.2 mg/dL) respectively. In contrast to pioglitazone® (100.0 ± 2.2 mg/dL), compound 5 showed significantly lower anti-hyperglycemic effect (44.5 ± 2.2 mg/dL) (*p* < 0.05) (Figure 4).

Hypoglycemic activity using sucrose loaded model (SLM)

All the chemical entities under investigation exhibited hypoglycemic activity when measured using the sucrose-loaded model (SLM). Compounds 1 and 3 demonstrated significantly higher anti-hyperglycemic activity after 1 h (78.5 ± 1.5 and 81.1 ± 1.8 mg/dL) respectively than other compounds, although lower than pioglitazone® (100 ± 2.1 mg/dL). Compound 5 exhibited significantly lower anti-hyperglycemic activity (36.4 ± 1.5 mg/dL). Compounds 1 and 3

demonstrated significantly higher anti-hyperglycemic activities (79.4 ±1.4, 84.8 ±1.3 mg/dL) compared to other compounds after 2 h. Also, compounds 1 and 3 demonstrated significantly higher hypoglycemic activity (81.3 ± 1.7, 85.1 ± 1.2 mg/dL) respectively compared to other compounds after 3 h (Figure 5).
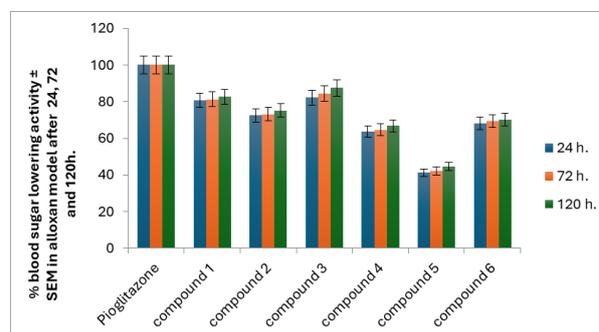

Figure 4: Anti-hyperglycemic activity of tested compounds 1-6 in alloxan-induced diabetic rats. *P* < 0.05 vs. pioglitazone. Values are presented as mean ± SEM
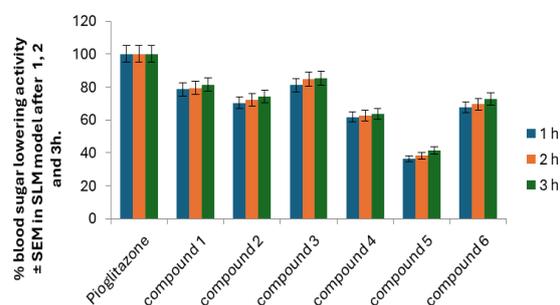

Figure 5: Anti-hyperglycemic activity of tested compounds 1 - 6 by sucrose loaded model. *P* < 0.05 vs. pioglitazone. Values are presented as mean ± SEM

Anticancer activity

When tested against human colon cancer cell lines (HT-29), lung cancer cell lines (A549), and prostate cancer cell lines (DU145), all the chemical entities demonstrated significant anticancer activity (Figure 6). When compared to doxorubicin® (6.52 mol/L), compounds 5 and 6 demonstrated the highest activity against a human colon cancer cell line (HT-29) with IC50 values of 6.38 and 7.82 mol/L compared to other compounds. In contrast, compounds 1, 2, 3, 4, and 6 demonstrated significantly lower efficacy (46.46, 23.64, 68.82, 20.38, and 21.51 mol/L, respectively), compared to doxorubicin® (6.49 mol/L). Furthermore, compound 5 exhibited higher activity against the human prostate cancer cell line (DU145), with an IC_{50} of 7.68 mol/L than doxorubicin® (6.83 mol/L) (Figure 6).
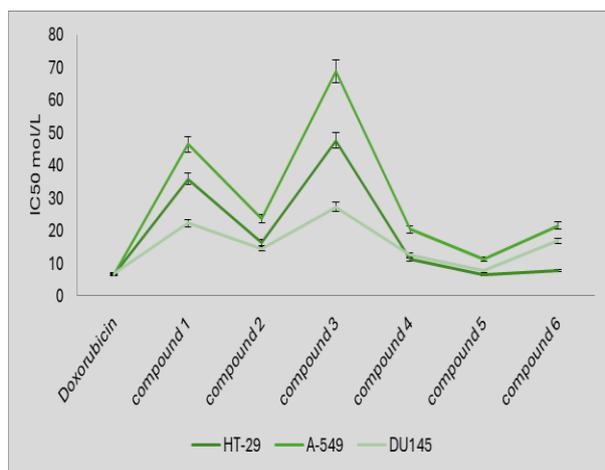
Figure 6: Anticancer activity of tested compounds 1 – 6. $P < 0.05$ vs. doxorubicin; n = 6. Values are presented as mean ± SEM

DISCUSSION

One of the biggest obstacles to drug development is polypharmacology, which creates new opportunities for the systematic design of new chemical entities that are less harmful yet highly effective [20]. The produced compounds (1 - 6) have been examined in these investigations for their anti-cancer, anti-hyperglycemic, analgesic, anti-inflammatory, and anti-microbial properties. These compounds have distinct actions depending on their functional groups and structure. In comparison to benzotropene, compounds 1 and 3 have strong anti-parkinsonian action. The substitution of NH_2 at R^1 and R^3 positions of compound 1 improved the antiparkinsonian activity. Compound 2 differed from compound 1 due to the presence of CH_3 at the R^1 position while other groups were the same. As a result, the anti-parkinsonian activity of compound 2 was lower than compound 1. Thus, OH at R^1 and substitution with an amine group (-NH_2) at both R^1 and R^3 were responsible for improved anti-parkinsonian activity. Structure-activity relationships based on these results indicated that substitution of pyrimidine compounds 1 and 3 have high anti-parkinsonian due to the presence of electron-donating moiety hydroxyl group (OH) and/or amino groups (NH_2).

Benzotropene is a synthetic tertiary amine and earlier studies on structure-activity relationships of benzotropene revealed that benztropine derivatives with the presence of a chlorine substituent in the para position in one of the phenyl rings produce an increased potency for dopamine uptake inhibition as well as a decreased inhibition of serotonin and norepinephrine [21]. In a similar study, a new series of poly-fused pyrazolothieno-pyrimidine

derivatives were synthesized and the results revealed that compounds 4, 7, 9, and 14 showed higher anti-parkinsonian activity nearly equal to that of benzotropene. This effect was attributed to the presence of hydroxy pyrazole moiety in compounds 4 and 7, aromatic ketone in compound 9 and secondary amine in compound 14 [22]. This corroborates the findings of this present research that the presence of amine in the ring structure as seen in compound 3 accounted for the improved anti-parkinsonian activity.

In investigating the anti-inflammatory activity of the chemical entities, compound 5 exhibited significantly higher anti-inflammatory activity compared to compounds 1, 2, 4, and 6 while compound 3 had the least anti-inflammatory activity due to the presence of CN group at the R^2 position relative to compound 4 and 6 which were shown to possess higher analgesic activity. Furthermore, the presence of the amine group (-NH_2) at the R^3 position resulted in reduced anti-inflammatory activity (as seen in compound 3). Also, compounds 4 and 6 have high analgesic due to the presence of withdrawing groups such as benzoyl (-COC_6H_5), (phenyl) and/or ethoxy carbonyl (-COOEt). Several studies have revealed that pyrimidines and fused pyrimidines, are integral parts of DNA and RNA and play an essential role in several biological processes [23]. Many of these derivatives were found to possess a variety of pronounced activities such as anti-inflammatory and analgesic which have been linked to the presence of a wide range of functional groups located in the parent ring structures [24]. The anti-inflammatory activity of ring substitutions had been studied alongside analgesic effect in an earlier study and the results revealed that fused pyrimidines have the ability to inhibit the expression and activity of key inflammatory mediators such as prostaglandin E2, inducible nitric oxide synthase (iNOS), tumor necrosis factor-α (TNF-α), nuclear factor kappa B (NF-κB), as well as chemokines and cytokines [25].

Compounds 5 (CN at R^2) and 6 (COOEt at R^2) exhibited high anti-microbial activity also due to the presence of withdrawing groups (phenyl) and/or cyano (CN). However, the anti-microbial activity of the compounds was highly varied among the bacteria and fungi strains consistent with the findings of the previous study [22]. A similar study on the anti-microbial activity of fused pyrimidines revealed that compounds 7 and 14 exhibited good inhibitions against bacteria strains (*Proteus mirabilis*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Escherichia coli* and *Staphylococcus aureus*) as well as fungi

strains (*Candida albicans* and *Aspergillus fumigates*) attributed to the presence of hydroxy pyrazole moiety in compound 7, and secondary amine in compound 14 [22]. Although amine group was present at R^1 (compounds 3 and 4), substitutions at the R^2 position accounted for the improved anti-microbial activity in this study. Furthermore, all the investigated compounds exhibited hypoglycemic lower than pioglitazone in both alloxan and sucrose-loaded models with compounds 1 and 3 inducing stronger effect compared to other compounds due to the presence of OH and CH_3 groups at R^1 and R^3 as well as amino ($-NH_2$) groups at R^1 and R^3 respectively. The presence of CH_3 group at the R^1 and R^3 positions (compound 2) resulted in a lower hypoglycaemic effect. Thus, compounds 1 and 3 exhibited high hypoglycemic effect due to the presence of donating hydroxyl group (OH) and/or amino groups (NH_2). In a similar study, new chemical entities of pyrimidine derivatives were investigated and the results revealed that compounds 8, 9 and 14 had the highest hypoglycemic activity due to the presence of ketonic moiety in compounds 8 and 9 and secondary amine in compound 14 [22] which is in tandem with this study.

The anticancer properties of these entities were also evaluated and the results revealed that compounds 5 (CN at R^2) and 6 (COOEt at R^2) exhibited higher activity compared to other compounds. Furthermore, compound 5 had higher anticancer activity than doxorubicin against human colon cancer cell lines (HT-29), lung cancer cell lines (A549), and prostate cancer cell lines (DU145) attributed to the presence of withdrawing groups as (phenyl) and/or cyano (CN). Several chemical processes play key roles in cancer cell death, and numerous anticancer therapies have undergone several structural modifications to improve efficacy. Among the reported medicinal attributes of pyrimidines, anticancer activity is the most extensively reported. The anticancer potential of pyrimidines in fused scaffolds has also been investigated resulting in a significant number of patents [26]. These patents were made due to modification of the parent pyrimidine scaffold.

CONCLUSION

Compounds **1** and **3** exhibit significant anti-parkinsonian, anti-inflammatory and hypoglycaemic effect while compound **6** exhibits significant anti-microbial, anti-fungi and anti-cancer effects. Thus, future studies into novel leads should incorporate these moieties for further experimental and clinical trials.

DECLARATIONS

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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