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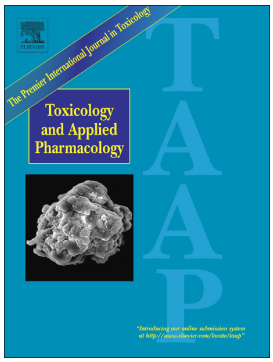

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Pharmacological and physicochemical profile of arylacetamides as tools against human cancers

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ABSTRACT

Arylacetamides are widely used as synthetic intermediates to obtain medicinal substances. This work evaluated *in vitro* antiproliferative activity of ten 2-Chloro-*N*-arylacetamides on human normal and cancer cells and detailed *in vivo* toxicological and anticancer investigations. Initially, cytotoxic colorimetric assays were performed using tumor lines, peripheral blood mononuclear cells (PBMC) and erythrocytes. Compounds **2**, **3** and **4** were tested for acute toxicity (50, 150 and 300 mg/kg) and for subacute antitumoral capacity in HCT-116 colon carcinoma-bearing xenograft mice for 15 days at25 mg/kg/day. Most compounds revealed cytotoxic action on tumor lines and PBMC, but activity on human erythrocytes were not detected. Molecular dipole moment, lipophilicity and electronic constant of aryl substituents had effects upon *in vitro* antiproliferative capacity. More common *in vivo* acute behavioral signals with compounds **2**, **3** and **4** were muscle relaxation, reduction of spontaneous locomotor activity and number of entries in closed arms and increased number of falls andtime spent in open arms, suggesting diazepam-like anxiolytic properties. Decrease of grabbing strength and overall activity were common, but palpebral ptosis and deaths occurred at 300 mg/kg only. Compounds **2** and **3**reducedcolon carcinoma growth (21.2 and 27.5 %, respectively, $p < 0.05$) without causing apparent signals of organ-specific toxicity after subacute exposure. The structural chemical simplicity of arylacetamides make them cost-effective alternatives and justifies further improvements to enhance activity, selectivity and the development of pharmaceutical formulations.

Keywords: colon carcinoma; xenograft model; physiological parameters; anxiolytic-like effects; behavioral animal.

1. Introduction

Cancer is a leading cause of death worldwide and the number of new cases is expected to increase considerably over the next decades, according to World Health Organization (WHO, 2017). There are many types of cancer treatment and the best selection depend on the cancer type and stage (Pazdur et al., 2002). Among them, chemotherapy is one of the most important and recommended treatments. However, several drawbacks, like drug resistance and adverse side effects, habitually limit adherence and treatment success (Carlotto et al., 2013; Willyard, 2016; Rapoport, 2017). On the other hand, these drawbacks have stimulated the search for new and secure anticancer drugs.

In this context, 2-Chloro-*N*-arylacetamidesbelongs to a chemical class widely used as synthetic intermediates to obtain bioactive substances for medicinal and chemical purposes. Chloroacetamides produce pharmaceutical intermediates during organic synthesis and are used as preservative of shampoos, cutting oils, plastics, coatings slab and shower gel (Amrutkar et al., 2012; Jain et al., 2013; Harkovet al., 2013). Reports have also demonstrated their potential as herbicidal, antimicrobial action against filamentous fungi (*Aspergillus niger*), yeasts (*Candida albicans*), bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*), and on drug-sensitive strain of *Mycobacterium tuberculosis* (Hamm and Speziale, 1956; Marco-Contelles and Gómez-Sánchez, 2005; Katke et al., 2011; Aschale et al., 2012).

However, to the best of our knowledge, their antiproliferative activity has not been evaluated so far. Studies on the mechanism of action of chloroacetanilides have indicated that the biological activity of this class could be attributed to its ability to alkylate important bionucleophiles (Jablonkai, 2003; Helleday et al., 2008; Singh et

al., 2011; Swift and Golsteyn, 2014). These features make chloroacetanilides an interesting subject of study as potential anticancer agents. Thus, in this work, we evaluated the *in vitro* antiproliferative activity of ten 2-Chloro-*N*-arylacetamides, differing in aromatic substituents, against human normal and cancer cells, Subsequently, *in vivo* toxicological investigations and analysis of anticancer action using a xenograft model of human carcinoma were performed to assess chemotherapeutic applications and toxic profile.

2. Material and Methods

2.1 Chemistry

Compounds **1-10** were synthesized (Table 1) and fully characterized by their melting points and IR, ^{1}H and ^{13}C NMR spectra as described by Lavorato et al. (2017).All compounds were solubilized in sterile dimethylsulfoxide (DMSO, Vetec, Brazil).

2.2 Cell culture and animals' facilities

Human leukemia (HL-60), ovarian (OVCAR-8), glioblastoma (SF-295), colon (HCT-116), andliver (HEPG-2) tumor lines andperipheral blood mononuclear cells (PBMC)were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin, at 37°C in a 5% CO_2 atmosphere (Shel Lab CO_2 Incubator, USA).

For PBMC isolation, heparinized human blood samples (from healthy, non-smoker donors who had not taken any drug for at least 15 days prior to sampling, aged 18-35 years old) were collected. Then, PBMC were isolated by the standard method of density-gradient centrifugation over Ficoll-Hypaque (Cultilab, Campinas,

Brazil). After some days, extra blood collection was performed and a suspension of red blood cells (2 %) was prepared. All studies were executed in accordance with Brazilian guidelines (Law 466/2012, National Council of Health), the Declaration of Helsinki and with the Universal Declaration on Bioethics and Human Rights of UNESCO.

For *in vivo* studies, Swiss (*Mus musculus*) andCB17 severe combined immunodeficiency (SCID) female mice were obtained from the animal facilities at Universidade Federal do Piauí (UFPI, Teresina, Brazil) and at Fundação Oswaldo Cruz (FIOCRUZ, Salvador, Brazil), respectively. Swiss mice were kept in ventilated racks (Alesco^{TM}, Brazil), while CB17-SCID animals were maintained in well-ventilated sterile cages (Tecniplast^{TM}, Germain), according to international standards for production and maintaining of germ-free animals. All animals were housed under standard conditions of light (12:12 hlight/dark cycle) and temperature (22 ± 1°C), with access to sterile commercial rodent stock diet (Nutrilabor, Campinas, Brazil) and water *ad libitum*. All procedures were approved by the Committee on Animal Research at FIOCRUZ (#006/2015) and UFPI (#202/2016) and followed Brazilian (*Colégio Brasileiro de Experimentação Animal* - COBEA) and International rules on the care and use of experimental animals (Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes).

2.3 Cytotoxicity analysis

The cytotoxic action was assessed by colorimetric assays after 72 h exposure. Cell proliferation was determined spectrophotometrically using a multiplate reader (DTX 880 Multimode Detector, Beckman Coulter). Control groups (negative and

positive) received the same amount of solvent (DMSO 0.1%). Doxorubicin (0.005 - 5 µg/mL) was used as positive control.

2.3.1 Antiproliferative assays with human tumor cells

The cytotoxicity on HL-60, OVCAR-8, SF-295, HCT-116 and HEPG-2 was determined by the MTT assay (Mosmann, 1983), which determines the ability of living cells to reduce the yellow dye 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-*2H*-tetrazolium bromide (MTT) to a purple formazan product. Line cells were plated in 96-well plates (0.3-0.7 x 10^5 cells/well) and incubated to allow cell adhesion or equilibration (suspension cultures). Twenty-four hours later, compoundswere added to each well (0.2 - 10µg/mL). After 69 h of incubation, the supernatant was replaced with fresh medium containing 10% MTT, and the cells incubated for an additional 3 h. The plates were centrifuged, formazan product was dissolved in DMSO and absorbance was read at 595 nm.

2.3.2 Antiproliferative study with human normal peripheral blood mononuclear cells

All compounds were also investigated on human PBMC using the Alamar Blue™ assay. PBMC were washed and resuspended (3 x 10^5 cells/mL) in supplemented RPMI-1640 medium plus 4% phytohemagglutinin for growth stimulation. PBMC were then plated in 96-well plates (3 x 10^5 cells/well in 100 µL of medium). After 24 h, compounds dissolved in DMSO were added to each well (0.2 - 25µg/mL) and cells were incubated for 72 h. Twenty-four hours before the end of the incubation, 20 µL of resazurin (Alamar Blue™) stock solution (0.156 mg/mL) (Sigma Aldrich Co., USA) were added to each well. The absorbance was read at 570 and 595 nm and the drug effect was expressed as the percentage of the control (Ferreira et al., 2015).

2.3.3 Hemolytic assay

Molecules were tested for hemolytic activity according to Santos et al. (2010) at 250µg/mL in 96-well plates during 60 min at room temperature (25^{o}C) using suspension of human erythrocytes (2 % in 0.85 % NaCl containing 10 mM $CaCl_2$). After centrifugation, hemoglobin levels in the supernatants were determined at 540 nm.

2.4. Acute toxicity and behavior analysis

Taking into consideration to minimize pain and suffering as well as ensuring the robustness and reproducibility of the experiments, it was adopted a methodology recommended by the acute toxic class method - Guideline 423 – described in the "Guideline for Testing of Chemicals" from OECD to evaluate the acute toxicity(OECD, 2001). It described that testing in one sex (usually females) is now considered sufficient because, although there is little difference in sensitivity between the sexes, in those cases where differences are observed females are generally slightly more sensitive. Since the compounds **2**, **3** and **4** were not tested previously, and their toxicity was not described yet, the initial dose administered to animals was 300 mg/kg. It is important to note that before administrations, all animals were acclimatized for 5 days. Administrations were performed and mice were observed for 14 days. Negative groups received DMSO 5% in distilled water since compounds were dissolved in pure DMSO.

Following the administration, the animals were fed restricted for 2 hours and observed after 60 min and 24 h. Thereafter, animals were observed daily until the 14th day. According to the daily Hippocratic screening, the following signs were

assessed: general activity, irritability, touch response, response to tail clamping, writhing, righting reflex, grip strength, auricular reflex, corneal reflex, tremors, convulsions, ptosis, piloerection, cyanosis, and death. It was also evaluated the variation of body weight, food and water consumption, and production of excretions (urination and defecation) using metabolic cages (Lucio et al., 2000). Due to the occurrence of deaths, it was proceeded a new administration at lower doses according to OECD 423. Doses observed the limit of 0.1 mL/10 g of body weight. Range of the LD_{50} was estimated according to the Globally Harmonized System (GHS) (Brazil, 2013).

For behavior investigations, experiments were performed as described below. Diazepam (2 mg/kg, oral by gavage) was administered as standard drug. Doses were chosen base on the toxicity studies and OECD guidelines. After each animal, the cleaning of the field was performed with a paper towel humidified with alcohol 70 % to remove excreta left by prior animals. All analyzes were conducted with each animal singly.

2.4.1 *Open field test*

The exploratory activity was verified using an open field made of acrylic (transparent walls and black floor, with dimensions of 30 x 30 x 15 cm) divided into 9 quadrants and based on the model described by Archer (1973) and Araújo et al. (2017). Thirty minutes after the treatment, animals were placed in the center of the open field. Afterwards, the number of intersections or crossings with four legs (spontaneous locomotor activity - SLA), number of self-cleaning behavior (grooming) and number of lifting without lean against the walls (rearing) were accounted for 5 minutes.

2.4.2 Rota rod test

The test route rod assesses the degree of muscle relaxation or motor incoordination induced by bioactive substances (Araújo et al., 2017). Each mouse was placed with all four feet onto a bar of 2.5 cm diameter, 25 cm high from the floor, in a rotation of 17 rpm for a period of 3 minutes. The duration of permanence in the swivel bar, in seconds (s), and the number of falls, with three renewals at maximum, was recorded.

2.4.3 Elevated plus maze test

The elevated plus maze apparatus consists of two open arms (30 x 5 cm) and two closed arms (30 x 25 x 5 cm) crossed perpendicularly. In this frame, the animal is placed 60 cm above the ground exactly on the intersection of the arms (central platform, 5 x 5 cm) with its head turned to the entry of closed arms (Lister, 1987). The animals were placed on the intersection of the arms 30 min after treatment and observed for 5 minutes. The parameters quantified in this test were number of entries into the open arms (NEOA) and time spent in the open arms (TSOA).

2.5 In vivo xenograft assay with human colon carcinoma

HCT-116 cells were maintained in supplemented RPMI-1640 medium, counted in Neubauer chamber and subcutaneously implanted into the left hind axillary of CB-17mice (2×10^7 cells/mL/animal). On the next day, animals were randomly divided into five groups (n=12 each) and the substances (**2**, **3** and **4**) dissolved in DMSO 5% were intraperitoneally administered for 15 days at25

mg/kg/day. Negative and positive controls received DMSO 5% (i.p.) and 5-FU (15 mg/kg/day, i.p.), respectively.

On the 16thday, animals were anaesthetized with ketamine (90 mg/kg) + xylazine (4.5 mg/kg) for blood collection from each animal via retrorbital plexus (Waynforth, 1980) using sterile tubes and heparinize pipettes to determine the profile of circulating peripheral leukocytes and analyzed at 400x magnification in May-Grünwald-Giemsa-stained blood smears (two per animal) to obtain differential amount of white blood cells (WBC).The absolute count of a leukocyte subtype was calculated as the product of its respective differential percentage and total leukocyte count (Biermann et al., 1999).

Afterwards, all mice were sacrificed by cervical dislocation and tumors, livers, kidneys, hearts and lungs were dissected out, weighed and fixed in 10% formaldehyde for examination of size, color changes and hemorrhages. The inhibition ratio of tumor growth (%) was calculated as follows: inhibition ratio (%) = [(A - B)/A] x 100, where A is the average tumor weight in the negative control, and B is the average in each separately treated group.

2.6 Evaluation of intestinal motility

Female Swiss mice were randomly divided into 6 groups (n = 8 animals/group): negative control (DMSO 5%, i.p.), positive control for increased intestinal transit (Bisacodil, 10 mg/kg, oral), positive control for reduced intestinal transit (Atropine sulfate, 5 mg/kg, i.p.) and the substances (**2**, **3** and **4**) (50 mg/kg, i.p.). Thirty minutes later, animals received 0.3 mL of activatedcharcoal10 % in carboxymethylcellulose1.5 % by gavage. After additional 30 min, mice were euthanized by dislocation cervical and the small intestines were withdrawn (from the

pylorus to the beginning of the cecum). Outcomes were expressed as a percentage of the total length of the small intestine (Harrison et al., 2004) as follows: Intestinal Transit (%) = Distance journeyed by activated carbon/Total length of small intestine x 100.

2.7 Statistical analysis

Values of IC_{50} and their 95% confidence intervals were obtained by nonlinear regression using the GraphPad program (Intuitive Software for Science, San Diego, CA). Differences were evaluated by comparing data using one-way analysis of variance (ANOVA) followed by the Newman-Keuls test ($p < 0.05$). All *in vitro* studies were carried out in duplicate and represent independent biological evaluations.

3 Results

3.1 Acute toxicity and behavioral changes

The compound **2** reduced grabbing strength and overall activity at 150 mg/kg and increased defecation, decreased general activity and grabbing strength, and presence of palpebral ptosis at the highest dose (300 mg/kg) (Table 1). Two animals died at 300 mg/kg after 24 h. Compound **3**, which has bromine in its chemical structure, decreased overall activity (apathy and absence of body tonus), and all animals died at 300 mg/kg after 24 h exposure. At 150 mg/kg, death was not detected but decreasing of general activity was obvious. Compound **4** diminished general activity, corneal reflex and grabbing strength, and increased urination and defecation at 300 mg/kg, and one death was noticed. At 150 mg/kg, death was not detected but decreasing of overall activity and grabbing strength were apparent.

Insert **Table 1** here

The open field test (Fig. 1) revealed reduction in the number of crossings after administration of compound **3** at 150 mg/kg (23.8 ± 6.8) and compound **4** at 300 mg/kg (18.8 ± 4.3) when compared to the negative group (41.3 ± 6.9 crossings) (p < 0.05).

Insert **Figure 1** here

The elevated plus maze, an experimental model used to investigate the modulation of anxiety *status* and exploratory activity of animals, showed that compounds **2** at 300 mg/kg and **4** at 150 and 300 mg/kg increased TSOA (179.0 ± 16.7, 196.2 ± 15.3 and 269.0 ± 10.4 s) and reduced TSCA (121.0 ± 17.0, 103.8 ± 15.3 and 38.8 ± 9.0 s) in a similar way displayed with diazepam (199.6 ± 27.3 s and 125.5 ± 14.0 s), if compared to the negative control (100.2 ± 8.4 e 199.8 ± 8.4 s), respectively (p< 0.05, Table 2). Moreover, the compound **4** changed NEOA for values (1.0 ± 0.3 entries) lower than those seen in the negative control group (3.6 ± 0.4 entries, p< 0.05). Meanwhile, the total number of entries was reduced by compounds **3** (150 mg/kg: 3.2 ± 1.5) and **4** (2.2 ± 0.5 entries, p < 0.05).

Insert **Table 2** here

Compounds **2** (300 mg/kg: 2.8 ± 0.2) and **4** (150 mg/kg: 2.4 ± 0.2; 300 mg/kg: 2.8 ± 0.2 falls) increased number of falls in the rota rod device (Table 3) when compared to the negative control (1.0 ± 0.4) (p < 0.05), but only compound **2** at 300

mg/kg diminished permanence time in the swivel bar for 46.8 ± 10.6 s, a value nearly 4-fold lower than that found for negative group (173.8 ± 2.9 s).

Deaths and/or behavior changes were noted with doses of 150 and 300 mg/kg, we also analyzed single doses at 50 mg/kg as suggested by OECD (2001). At this dose, significant outcomes and deaths were not found when compared to negative control (details not showed).

3.2 In vitro cytotoxicity on tumor and normal cells and toxicity

A series of 2-chloro-*N*-arylacetamides (**1**-**10**) was evaluated for their cytotoxicity against five tumor cell lines. These compounds were obtained by the reaction of ten *para*-substituted aniline derivatives with 2-chloroacetic anhydride at room temperature (Lavorato et al., 2017). With exception of compounds **6** and **9**, all compounds displayed IC_{50} values ranging from 4.9 to 50.1 μM (Table 3). Compound **2** (R = Cl) was the most active against three of the five tumor cell lines studied in this work (HL-60, SF295 and HCT-116 cell lines). Additionally, compounds **3** (R = Br) and **4** (R = NO_2) were the only active substances against the most resistant cell line SF-295.

Interestingly, the compounds did not cause significant *in vitro* cytolytic action against human erythrocytes until the concentration tested (250μg/mL). On the other hand, most molecules were also cytotoxic on proliferating human PBMC, and IC_{50} values ranged from 3.4 to 7.6 μg/mL (Table 3).

Insert **Table 3** here

Physicochemical data, including the influence of van der Waals volume (vdWV), Hammet's electronic constant (σ), lipophilicity, and dipole moment and their relationship are described in Table 4 and Fig. 2, 3, 4 and 5. They are examined in the Discussion section.

Insert **Table 4** here

Insert **Figure 2, 3, 4** and **5** here

3.3 In vivo antitumor action and subacute effects

Compounds **2**, **3** and **4** were tested for *in vivo* antitumor activity using a xenograft model of colon carcinoma. Only compounds **2** and **3**significantly reduced tumor growth in 21.2 % (0.57 ± 0.02 g) and 27.5 % (0.53 ± 0.04 g) at 25 mg/kg/day (Table 5)when compared to the negative control (0.73 ± 0.04 g respectively, $p < 0.05$). Comparably, 5-FU, as positive control, also cause tumor reduction.

Insert **Table 5** here

Deaths or changes in relative weight of organs and in hematological parameters were not detected in arylacetamides-treated animals ($p > 0.05$) but livers increased in 5-FU-treated mice (6.18 ± 0.39 g, $p < 0.05$) in comparison with negative control animals (4.95 ± 0.14 g) (Table 6). Similarly, only animals receiving 5-FU revealed decreasing of erythrocytes, leukocytes, hemoglobin content and hematocrit levels ($p < 0.05$, Table 6).

Insert **Table 6** here

The compounds 2, 3 and 4 were not able to cause diarrhea. Moreover, they did not alter the intestinal transit (71.1 ± 2.3, 57.9 ± 4.6 e 72.2 ± 4.9 %, respectively) when compared to the negative group (67.3 ± 4.0 %). On the other hand, Bisacodil increased the distance travelled by activated charcoal for85.1 ± 3.7 % and atropine (muscarinic blocker) reduced the intestinal transit for 39.8 ± 4.7 % ($p < 0.05$).

4. Discussion

The interest in molecular modeling, combinatorial chemistry and other techniques of chemical synthesis in medicinal chemistry is responsible for the newest therapeutic agents against parasites and cancers, and to treatinflammatory, neurodegenerative and sensory disorders(Soares et al., 2009; Oliveira et al., 2013; Ferreira et al., 2015; Lopes et al., 2015; Araújo et al., 2016; Almeida et al., 2017). Despite some studies relating bioactivity of synthetic arylacetamides, this is the first work focusing on the antiproliferative properties of 2-chloro-*N*-arylacetamides. Subsequently, we also performed *in vivo* preclinical evaluation about their toxicological and antitumoral capacity using xenograft model of colon carcinoma.

Herein, synthetic arylacetamides presented different antiproliferative activity according to the tumor cell line tested and the nature of the aryl substituent at the *para* position. Compound **6** (R = OCH_3) and **9** (R = COOH) were considered inactive against all cell lines they were evaluated whereas only compounds **3** (R = Br) and **4** (R = NO_2) were active against all tumor cells. We also investigated the role of several physicochemical properties of the evaluated compounds, like lipophilicity, dipole moment and van der Waals volume, as well as electronic effects of the aryl substituent in antitumor potential of this chemical class. To this analysis, IC_{50} values in micromolar (µM) were used.

Although we could not clearly establish the effect of aryl substituent to antiproliferative activity of 2-chloro-*N*-arylacetamides, some aspects should be highlighted. The unsubstituted compound **1** was one of the least active compounds, indicating that the presence of a substituent at *para* position is a contributing factor to activity. Among *para*-substituted compounds, only **2** (R = Cl), **3** (R = Br) and **4** (R = NO_2) were active against glioblastoma SF-295 cells. These compounds present van der Waals volumes ranging from 157.61 to 166.65 $Å^3$. This may indicate that volume occupied by such compounds can be an important feature to the cytotoxic activity. Nevertheless, based only in this parameter, we would expect compound **5** (R = CH_3), whose van der Waals volume is 160.45 $Å^3$, it would be also active on SF-295 line. So, besides steric aspects, it is likely that the electronic effect of the aryl substituent may also interfere on the activity against this cell line. The electronic effect of a substituent can be measure by its Hammet's electronic constant (σ). A positive σ indicates that the substituent is an electron-withdrawing group, whereas a negative σ is related to an electron-donating group (Tavares et al., 2004). According to their σ values, chloro, bromo and nitro play an electron-withdrawing effect, whereas methyl group plays an electron-donating effect, which can contribute to explain the bioactivity differences of these compounds. Thus, this information indicates that the compounds with an electron-withdrawing substituent present a potential antiproliferative activity against glioblastoma SF-295 cells.

The antiproliferative activity of 2-chloro-*N*-arylacetamides against OVCAR-8 cell line can also be partially explained by Hammet's electronic constant of ring substituents (σ). We observed that the most potent compound against OVCAR-8 cell line, compound **4** (R = NO_2), presents a substituent with the highest and positive σ of the series, indicating its high electron-withdrawing character. On the other hand, the

least active compound **1** (R = H) have no substituent, so the σ value attributed to hydrogen is zero. Accordingly, we would expect compound **8** (R = SO_2NH_2), with the second highest σ value (0.57), to be more active against tumor lines. However, outcomes frustrate this possibility. Probably, this occurred because this compound has the lowest lipophilicity among active compounds (ClogP = 0.35). A compound with low lipophilicity is less able to cross cell membranes, which reduces its ability to act into tumor cells (Rutkowska et al., 2013). Although possessing a lower value of σ for bromine, compound **3** (R= Br) present higher cytotoxic potential than **8** (R = SO_2NH_2), probably due to its higher ClogP. Indeed, we found a negative correlation among IC_{50} values and σ e ClogP, as described in the following equation:

$$IC_{50} = - 9.2985\ ClogP – 27.7010\ \sigma + 59.8736\ (n = 6; R = 0.901)$$

The correlation model indicates that both physicochemical properties can influence the antiproliferative activity of these compounds against OVCAR-8 cells, increasing the activity or reducing IC_{50} values as the values of these two parameters increase. Although it is not a predictive model, it is in accordance with our qualitative analysis of active compounds in the present study. The high electron-donating character of methoxy group in compound **6**and the low lipophilicity of **9** (R = COOH) could explain the absence of activity of these compounds. However, based only on the physiochemical characteristics, we could not find a logical reason for the unexpected inactivity of compounds **2** (R = Cl) and **7** (R = $COCH_3$) against this cell line.

On the other hand, compound **2** (R = Cl) presented a potent antiproliferative effect against HL-60 cells, with an IC_{50} significantly lower than the IC_{50} of the other compounds. We were not able to propose any structure-activity relationship regarding this cell line with the data available thus far. In this case, we cannot

establish a correlation among the antiproliferative profile of active compounds against HL-60 cells and their electronic and lipophilicity features. Compounds **4** (R = NO_2), **7** (R = $COCH_3$) and **8** (R = SO_2NH_2), with electron-deficient aromatic rings, present different activity profiles, since **4** and **8** are almost twice active than **7**. Compounds **4** and **8** are also more active than **3** (R = Br), which is the one whose physicochemical properties presented in **Table 4** most resemble the properties of compound **2**, the most active compound against this cell line. Although the lipophilicity of **2** can justify its better activity compared to **4** and **8**, the same property cannot explain why **3** has a higher IC_{50} value than **5** (R = CH_3), even though it has an electron-deficient aromatic ring.

The electronic constant of the ring substituent (σ) seems also to affect the antiproliferative activity of these compounds against HCT-116 cells. We observe a tendency towards the increase of the activity as σ increases, i.e., as the ring electronic density decreases. However, compounds **2** (R = Cl) and **3** (R = Br) do not follow this trend. We believe that the high lipophilicity of **2** and **3**, represented by their high ClogP values, can be a positive contribution to their antitumor activity.

Regarding the activity against HepG2 cell line, the compound's dipole moment seems to influence the antiproliferative action of 2-chloro-*N*-arylacetamides, increasing their activity as its value increases. Compounds **4** (R = NO_2), **7** (R = $COCH_3$) and **8** (R = SO_2NH_2) present the highest values of dipole moment among the evaluated compounds and were also the most active against HepG2 cells, whereas compound **1** (R = H), the least active against HepG2, displayed the lowest dipole moment.

In MTT assays, compound **9** (R = COOH) was inactive against all tumor cell lines. Interestingly, compound **10** (R = $COOCH_2CH_3$), the ethyl ester of **9**, was active

against two lines only. Since bioassays were run at pH~7.4, compound **9** was in its ionized form. Then, its antiproliferative potential may be reduced since it cannot permeate membranes and reach inside tumor cells (Lima et al., 2007). If this is true, the low antiproliferative activity of **10** may be explained by its *in-situ* hydrolysis and conversion to **9**.

The initial analysis of structure-activity relationship indicates that physicochemical features work jointly to affect antitumor effects of this series. By the way, the compounds that stood out in the studies – **2** (R = Cl), **3** (R = Br) and **4** (R = NO_2) – have lipophilic character and lower van der Waals volumes. These qualities contribute to the ability to cross cell membranes and to accumulate inside tumor cells. Moreover, these three compounds have electron-withdrawing aromatic substituents and a dipole moment that contribute to accentuate electrophilic properties of chloroacetamide derivatives. Since biological activities of this chemical class have been attributed to the ability to alkylate nucleophiles, like protein and nucleic acids (Jablonkai, 2003; Helleday et al., 2008; Singh et al., 2011; Swift and Golsteyn, 2014), these findings suggest that the antiproliferative activity described here may also be attributed to the same mechanism of action.

In the present work, only a small set of compounds was tested in order to assess whether this chemical class has a potential use as antitumor agents. So, the small dataset limits the performance of conclusive QSAR analysis. Moreover, the antiproliferative activity was determined against tumor cells, not against an isolated specific target. This could explain why we have not found a linear correlation between the activity and a specific physicochemical property (Scior et al., 2009). In this case, the activity-correlated properties lipophilicity and van der Waals volume are likely to influence the antitumor action by affecting the ability of compounds to

permeate cell membranes to reach this target. On the other hand, the properties dipole moment and electronic effect of aromatic substituent may have a greater influence on the compound recognition by macromolecules inside cell, its interaction with its molecular target and its alkylating profile, directly affecting the biological activity.

When assayed on human erythrocytes, none of the compounds showed critical lytic action, suggesting cytotoxicity is not related to the direct action on cellular membrane disruption. On the other hand, only compound **5** (R = CH_3) was non-toxic towards PBMC cells (IC_{50} > 25 µg/mL). So, most compounds did not reveal selective cytotoxicity only upon tumor cells. Interestingly, **5** is the only evaluated compound with an electron-donating aromatic substituent (**Table 4**), which contributes to reduce the electrophilicity of the compound and consequently its ability to alkylate bionucleophiles. This suggests that the cytotoxicity may also be modulate by the electrophilic character of the compound in the same way as the antiproliferative activity previously discussed.

Given that the toxic profile of a probable drug must be part of early steps for the development of new medications (Brazil, 2013; Ferreira et al., 2015; Araújo et al., 2018), we also analyzed the acute toxicity of the compounds that stood out to determine toxic effects and to establish safe dosages for subsequent pharmacological studies.

According to the guideline OECD 423 and based on the Globally Harmonised System, compounds **2**, **3** and **4** presented intermediate toxicity, since all of them are classified in the Category 3 (LD_{50} value: > 50 mg/kg < 300 mg/kg) (OECD, 2001). Indeed, studies have suggested that some 2-chloroacetamides present LD_{50} values ranging from about 30 to 300 mg/kg of body weight, and that such toxicity apparently

depends neither on routes of administration nor species-specific results (CDC, 2018; National Library of Medicine, 2018).

In the hippocratic screening study, the most common events were reduction of general activity and loss of grapping strength. These effects suggest central nervous system depressant activity despite loss of the righting reflex had not been observed. Such findings indicate these compounds may have central depressant action or selective sedative activity. The loss of grapping strength is an indication for skeletal muscle relaxant activity, which may be peripherally (at the neuromuscular junction) or centrally located (Carlini and Burgos, 1979; Kanjanapothiet al., 2004; Araújo et al., 2017). With this in mind, motor effects using a revolving bar were evaluated, and it was detected that compounds **2** (by reducing time on the revolving bars and increasing falls at 300 mg/kg) and **4** (by increasing falls at 150 and 300 mg/kg) have a myorelaxant activity and cause light but significant psychomotor retardation. It is likely that motor effects are associated with ataxia caused by some2-Chloroacetamides (CDC, 2018).

Reduction ofcrossingswas noticed for compounds **3** and **4** suggesting modifications in SLA of treated animals, but none of the three compounds altered the number of groomings and rearings, indicating such compounds do not interfere in the exploratory activity of mice subjected to open spaces nor affect the motor coordination. On the other hand, compounds **2**, **3** and **4** reduced the NECA and compounds **2** and **4** increased TSOA (and reduced TSCA) in a very similar way seen for diazepam (standard drug).These conclusions were found using the plus maze test, which consists of a more specific anxiety model for the assessment of anxiolytic drugs with capacity to reduce the rejection animals present to walk to the open arms,

since this behavior is conditioned by the fear or stress in aversive environments (Walf and Frye, 2007; Neumann et al., 2011).

In an overview, the acute behavioral signals more commonly associated with compounds **2**, **3** and **4** with were muscle relaxation and reduction of locomotor activity in the revolving bar with consequent increase in the number of falls, decrease of NECA and increase of TSOA, suggesting sedative actions. Such actions surprisingly exhibit characteristics of anxiolytic properties related to the diazepam, mainly for compound **2** (R = Cl) and **4** (R = Br). These behavioral data confirm the reduction of general activity, coordination of the motor system (grabbing strength) and muscle tonus from Hippocratic screening tests (Walf and Frye, 2007; Almeida et al., 2012).

Ash (2004) demonstrated that the LD_{50} of some 2-chloroacetamides on mice is around 100mg/kg body weight, which could justify, at least in part, the presence of toxicity signs at 150mg/kg. Behavioral effects analyzed in glyphosate-based herbicide-treated mice showed impairment effects upon the central nervous system probably due to alterations in neurotransmission pathways that participate or regulate locomotor activity, anxiety and memory involving GABAergic, dopaminergic, serotonergic and/or cholinergic systems (Baier et al., 2017).

Benzodiazepines, as diazepam and alprazolam, present a range of actions – sedative/hypnotic, anxiolytic, anticonvulsant and muscle relaxant – and most of them were found in arylacetamides-treated animals. It is likely such synthetic compounds do not activate $GABA_A$ receptors directly but, instead, are positive allosteric modulators of the effects of GABA and allow lower concentrations of this neurotransmitter to open the Cl^- channels, like most benzodiazepines. As a consequence of the enhancement of GABA's inhibitory activity caused by

benzodiazepines, the brain's output of excitatory neurotransmitters including norepinephrine, serotonin, dopamine and acetylcholine is reduced (Sigel and Steinmann, 2012; Miller and Aricescu, 2014). Further pharmacological investigations are in progress to confirm the mechanism(s) of action because this was not the main focus of this research.

Based on the absence of toxicity with doses of 150 mg/kg and considering that a drug has a good safety profile if its therapeutic index exceeds the value of 8-10 (Tamargo et al., 2015), we elected the dose of 25 mg/kg/day for efficacy assays. As *in vivo* models, xenograft animals are technically represented by athymic nude mice, severely compromised immunodeficient (SCID) mice. They have been extensively used to monitor tumorigenicity and tumor growth, can simulate the complexity of genetic and epigenetic abnormalities in human tumors and aid in the development of individualized molecular approaches (Morton and Houghton, 2007; Jung et al., 2014).

In the present study, we chose colon carcinoma as preclinical cancer model due to its epidemiological importance: a) colorectal neoplasm is the third mostly common occurring cancer in the world; b) causes about 694,000 deaths a year (10 % of all cancer deaths); c) presents wide geographical variation in incidence with rates varying ten-fold in both sexes, and d) 95 % of them are adenocarcinomas, especially in countries characterized by high or very high indices of development and/or income (two thirds of cases), which strongly demonstrates how certain lifestyle factors affect the risk of developing colorectal carcinomas (Ferlay et al., 2014; Mármol et al., 2017).

We showed that compounds **2** and **3** significantly reduced colon carcinoma tumor growth. We believe that their higher lipophilicities, represented by high Clog P values, has positive (but partial) role in this antitumor activity. Furthermore, the electronic constant of the ring substituent (σ) seems also to affect the antiproliferative

activity of the series against *in vitro* HCT-116 cells, being observed a tendency towards the increase of bioactivity as σ increases, i.e., as the ring electronic density decreases. However, compounds **2** (R = Cl) and **3** (R = Br) do not follow this trend, and *in vivo* tumor inhibition rates were equivalent ($p > 0.05$).

Some 2-chloro-*N*-arylacetamides behave as alkylating agents, especially those molecules containing sulfhydryl groups (Jablonkai and Hatzios, 1991; Jablonkai, 2003). It is also worth to note that classical classes of antitumor molecules, like nitrogen mustards, nitrosoureas and quinones causes cell death clearly associated with electronic and lipophilic properties (Hansch et al., 1972; Denny and Wilson, 1986; Gourdie et al., 1990; Driebergen et al., 1993).

Compounds tested *in vivo* neither caused macroscopical/morphological damage on key organs, affected intestinal transit nor altered figurative elements of the peripheral blood, which suggest that subacute exposure for 15 days at 25mg/kg/day neither promoted direct action on blood cells/hematopoiesis nor showed target organ toxicity, a great advantage if we take into consideration that most antineoplasic drugs clinically available cause myelosuppression (anemia, leucopenia with neutropenia), hepatotoxicity, diarrhea, and alopecia, besides cardiotoxicity, opportunistic infections, peripheral neuropatia, nausea, vomiting, anorexia, fatigue and tiredness (Carlotto et al., 2013; Ferreira and Pessoa, 2017;Rapoport, 2017;Nurgali et al., 2018).

In summary, most of 2-chloro-*N*-arylacetamide derivatives showed moderate to high antiproliferative action on tumor lines, cell toxicity on normal dividing leukocytes, and *in vivo* investigations demonstrated diazepam-like anxiolytic properties of compounds **2** and **4**, including muscle relaxation and reduction ofspontaneous locomotor activity, general mobility and muscle tonus.

Physicochemical characters as van der Waals volume, molecular dipole moment, lipophilicity and electronic constant may work together to alter the anticancer capacity, but the evaluation of more compounds of this chemical class is imperative to better understanding their structure-activity relationship. Compounds **2** and **3** reduced tumor growth in xenograft colon carcinoma-bearing mice without causing apparent signals of organ-specific toxicity after subacute exposure. The structural chemical simplicity of these arylacetamides make them cost-effective alternatives and justifies further improvements to enhance activity and selectivity and the development of pharmaceutical formulations.

Conflict of interest

The authors declare that there are no conflicts of interest.

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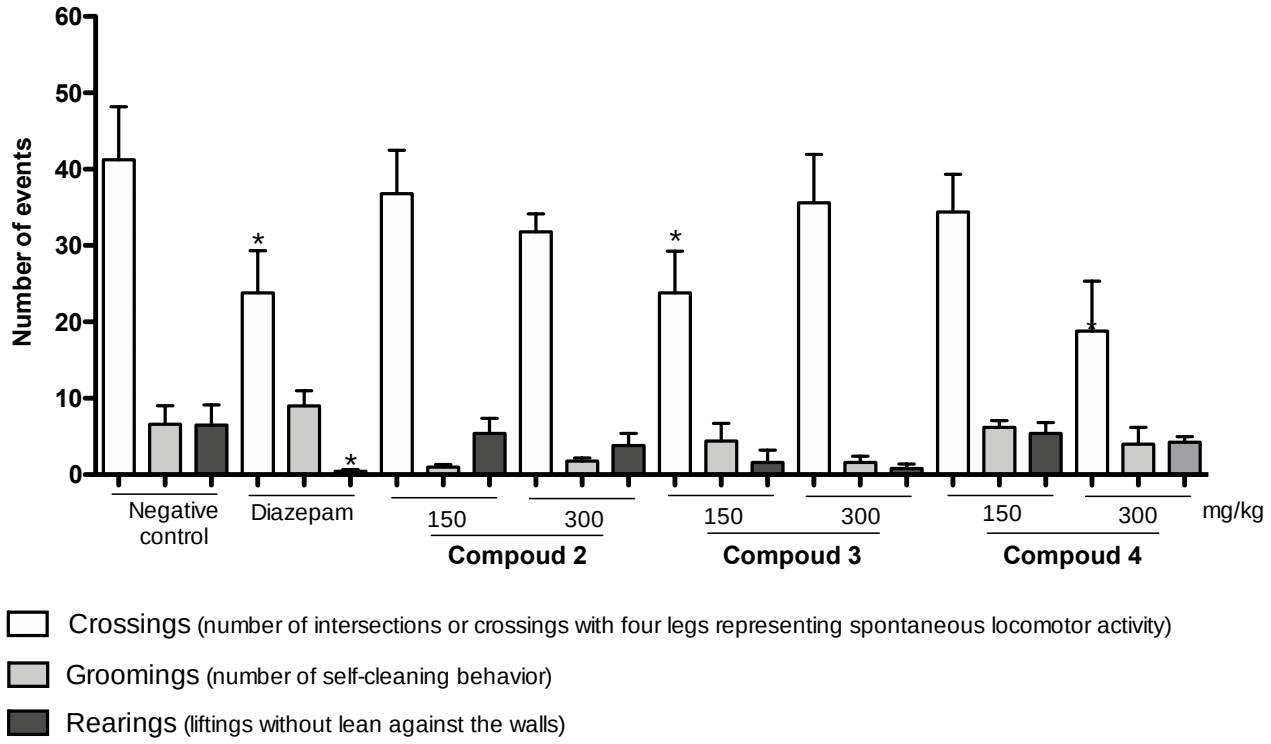


Fig. 1. Behavioral assessment of mice treated with synthetic arylacetamides. Results were expressed as mean ± S.E.M. (n = 5 animals/group). Negative control received DMSO 5 %. Positive control was treated with Diazepam (2 mg/kg/day). *$p < 0.05$ compared to the negative control by ANOVA followed by Student-Newman-Keuls.

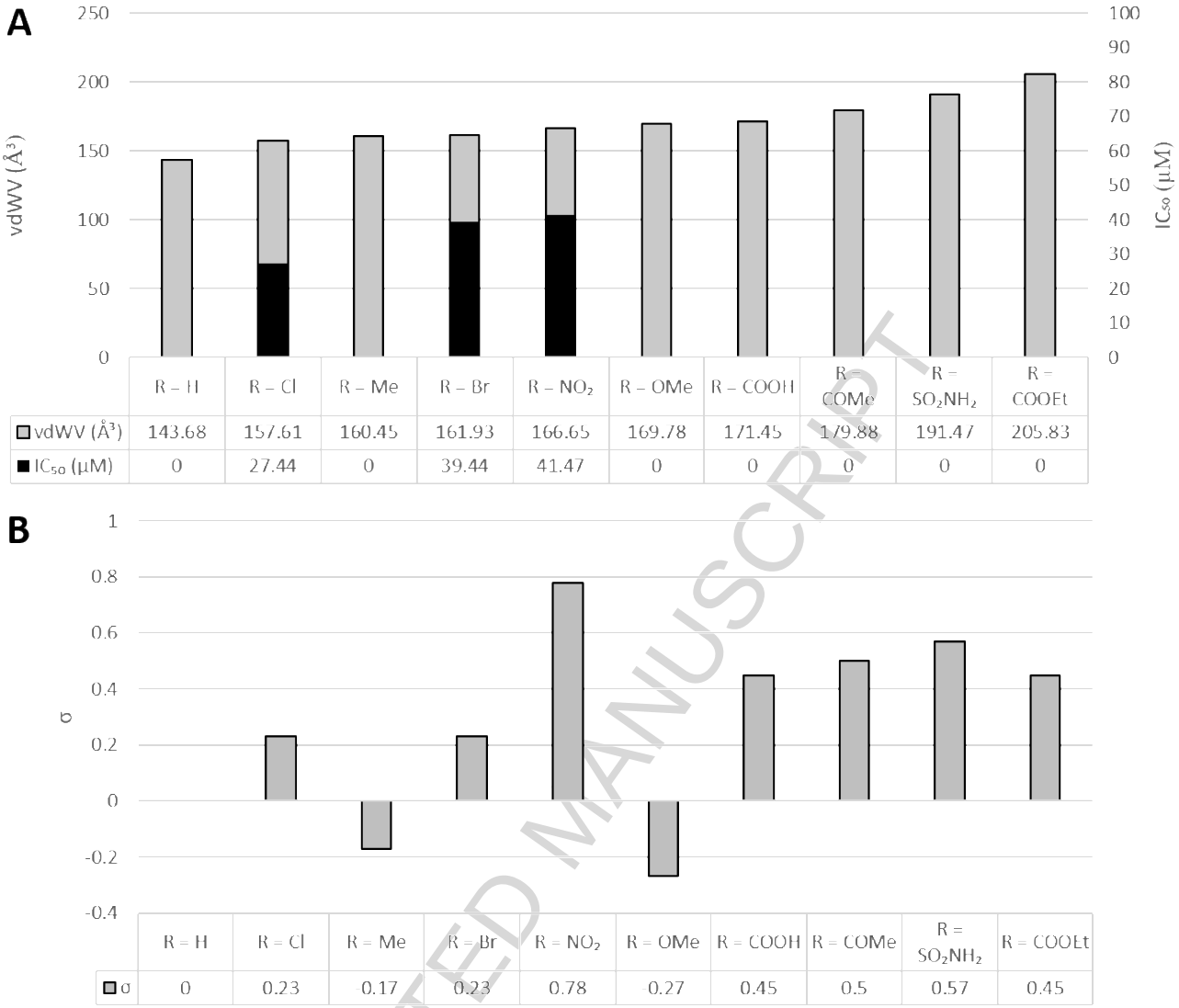
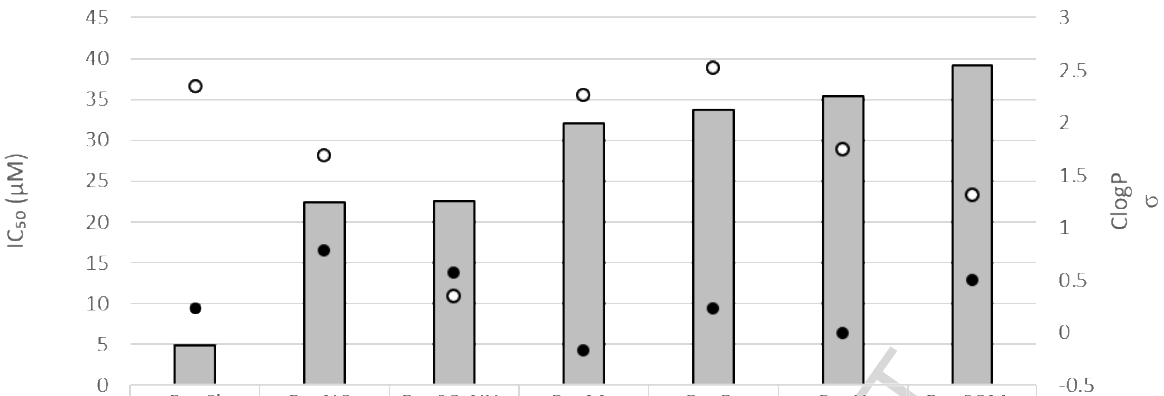


Fig. 2. The influence of van der Waals volume (vdWV) and Hammet's electronic constant (σ) on the antiproliferative activity of 2-chloro-*N*-arylacetamides against SF-295 cell line. A - vdWV (grey columns) and IC_{50}(black columns) values of active compounds against SF-295 arranging in ascending order of vdWV values B - σ values (grey columns) of active compounds against SF-295.



	R = Cl	R = NO_2	R = SO_2NH_2	R = Me	R = Br	R = H	R = COMe
IC_{50} (μM)	4.9	22.37	22.52	32.13	33.8	35.38	39.22
σ	0.23	0.78	0.57	0.17	0.23	0	0.5
Clog P	2.35	1.69	0.35	2.26	2.52	1.75	1.31

Fig. 3. The influence of Hammet's electronic constant (σ) and lipophilicity (ClogP) on the antiproliferative activity of 2-chloro-N-arylacetamides against HCT-116 cell line. A) IC50 (grey columns) and σ (black balls) values of active compounds against HCT-116 arranging in ascending order of IC50 values. B) ClogP values (grey columns) of active compounds against HCT-116.

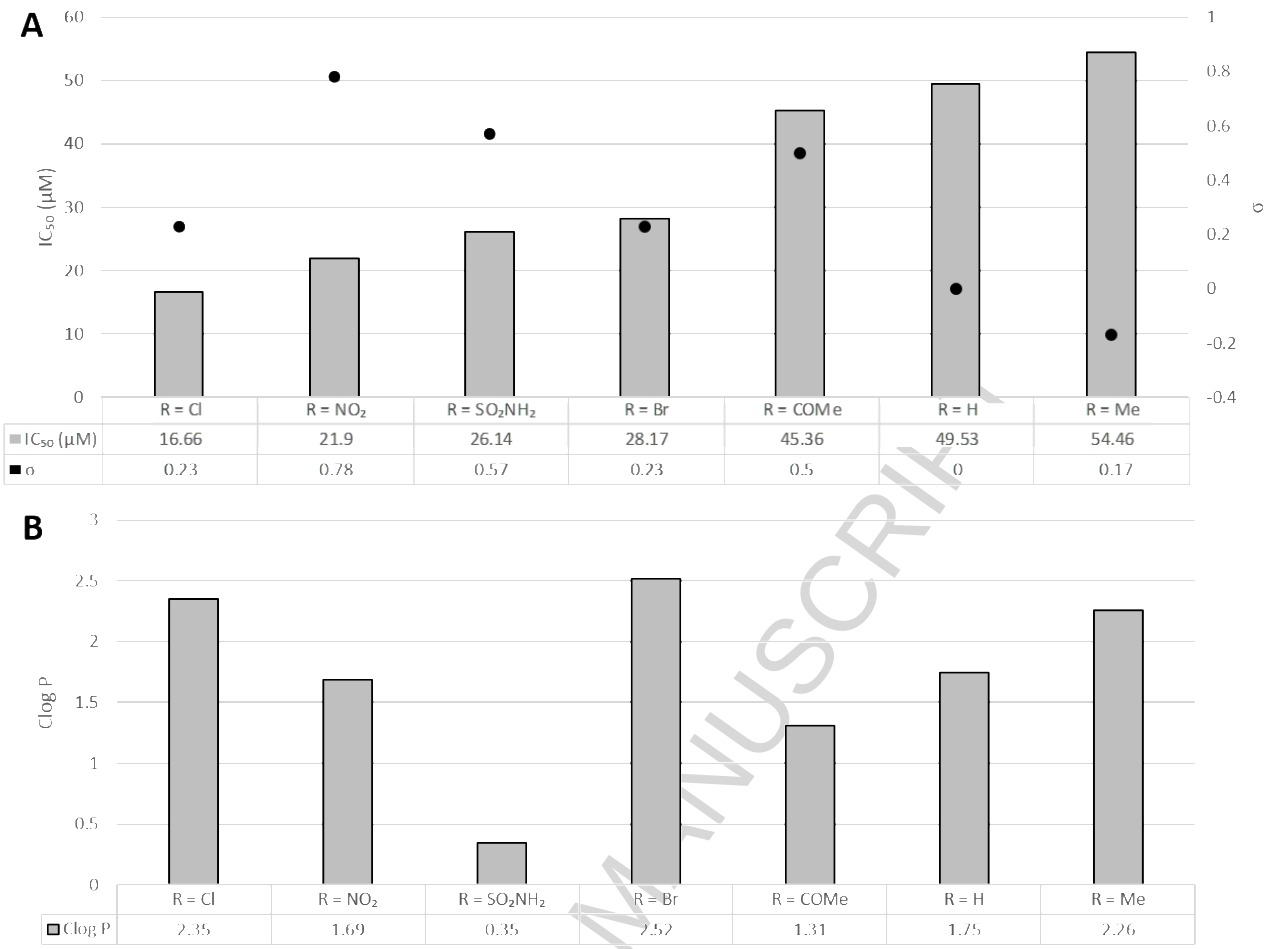
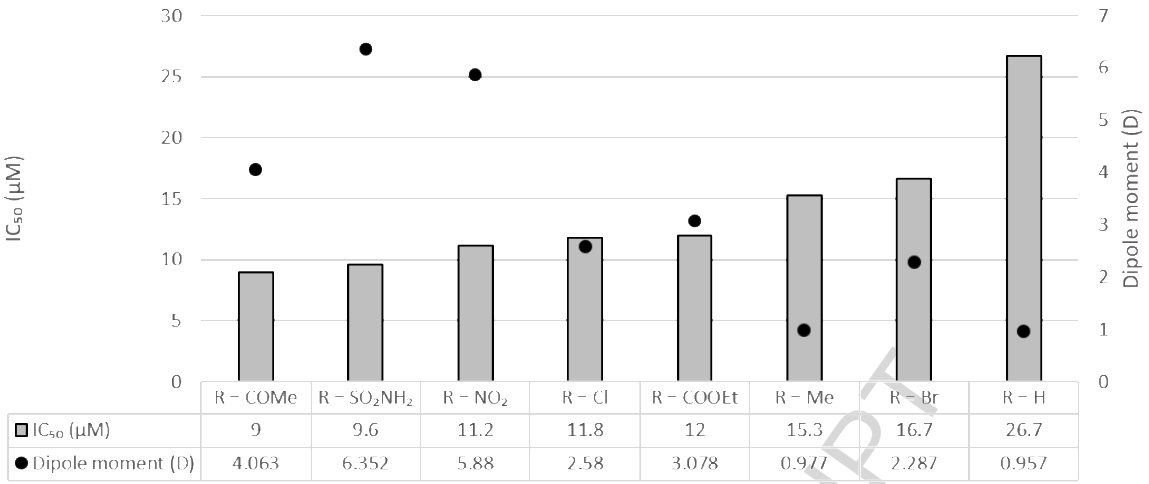


Fig. 4. The relationship among IC50 values (columns in grey) and Hammet's electronic constant (σ) (black balls) and lipophilicity (ClogP) (white balls) of active 2-chloro-N-arylacetamides against HL-60 cell line. The data is arranged in ascending order of IC_{50} values.



	R – COMe	R – SO_2NH_2	R – NO_2	R – Cl	R – COOEt	R – Me	R – Br	R – H
IC_{50} (μM)	9	9.6	11.2	11.8	12	15.3	16.7	26.7
Dipole moment (D)	4.063	6.352	5.88	2.58	3.078	0.977	2.287	0.957

Fig. 5. The relationship between IC50 (columns in grey) and dipole moment (balls in black) values of active 2-chloro-N-arylacetamides against HepG2 cell line. The data is arranged in ascending order of IC_{50} values.

Highlights

Most molecules revealed cytotoxicity and CI_{50} values from 4.9 to 50.1 μM.

Steric aspects, lipophilicity and substituents had effects on *in vitro* antitumoral action.

Single dose studies showed diazepam-like anxiolytic properties of compounds **2** and **4.**

In vivo signals of organ-specific toxicity were not detected after subacute exposure.

Compounds **2** and **3** reduced *in vivo* xenograft human colon carcinoma growth.

Table 1

Acute toxic effects of the synthetic arylacetamides **2**, **3** and **4** after intraperitoneal injection in Swiss mice.

Group	Dose (mg/kg)	Signs of toxicity		
		Survival	1 h	24 h
Negative control	-	5/5	-	-
Compound 2	150	5/5	-	Decreased grabbing strength and overall activity
	300	3/5	Increased defecation	Increased defecation, decreased general activity and grabbing strength, and presence of palpebral ptosis
Compound 3	150	5/5	-	Decreased overall activity
	300	0/5	Decreased grabbing strength and overall activity	Decreased overall activity, presence of palpebral ptosis, increased urination and defecation
Compound 4	150	5/5	Decreased overall activity	Decreased grabbing strength and overall activity
	300	4/5	Decreased overall activity and body tonus	Reduction of general activity, corneal reflex, and grabbing strength, and increased urination and defecation

Data from n = 5 animals/group. Negative control was treated with the vehicle used to dilute the drug (DMSO 5 %).

Table 2

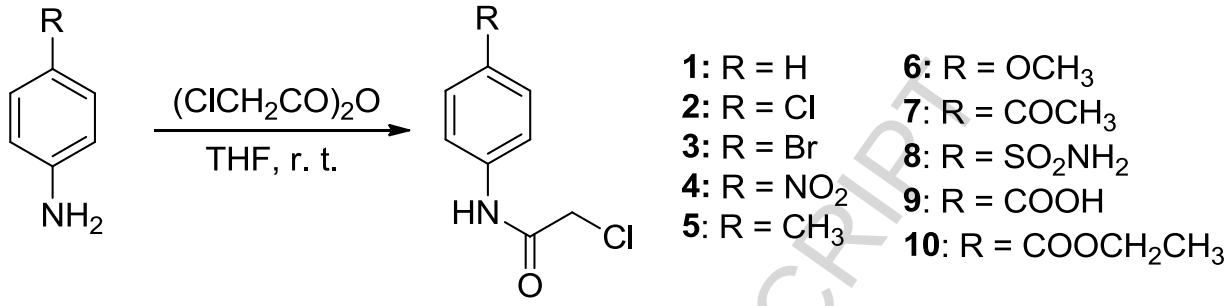
Effect of synthetic arylacetamides **2**, **3** and **4** on the number of entries in open arms (NEOA), number of entries in closed arms (NECA), time spent in open arms (TSOA), time spent in closed arms (TSCA), and total number of entries into open and closed arms determined by the elevated plus maze test, and on motor coordination analyzed by the rota rod test.

oup	**Dose** (mg/kg)	**NEOA**	**NECA**	**TSOA** (s)	**TSCA** (s)	**Total number of entries**	**Number of falls**	**T per in r b**
e control	-	3.6 ± 0.4	4.6 ± 0.4	100.2 ± 8.4	199.8 ± 8.4	8.2 ± 0.8	1.0 ± 0.4	173
epam	2	3.4 ± 0.9	3.2 ± 0.2*	199.6 ± 27.3*	125.5 ± 14.0*	6.6 ± 1.7	3.0 ± 0.5*	71
2	150	2.2 ± 0.4	1.8 ± 0.4*	137.0 ± 12.4	163.0 ± 12.4	4.0 ± 0.6	1.8 ± 0.4	160
	300	2.8 ± 0.4	1.8 ± 0.4*	179.0 ± 16.7*	121.0 ± 17.0*	4.6 ± 0.7	2.8 ± 0.2*	46.
3	150	2.0 ± 0.8	1.4 ± 0.7*	153.8 ± 57.8	182.8 ± 14.6	3.2 ± 1.5*	1.2 ± 0.7	157
	300	2.8 ± 0.6	2.4 ± 0.7	162.2 ± 36.3	172.3 ± 14.6	5.2 ± 1.2	1.6 ± 0.7	143
4	150	1.8 ± 0.4	2.6 ± 0.5	196.2 ± 15.3*	103.8 ± 15.3*	4.4 ± 0.9	2.8 ± 0.2*	144
	300	1.0 ± 0.3*	1.2 ± 0.2*	269.0 ± 10.4*	38.8 ± 9.0*	2.2 ± 0.5*	2.4 ± 0.2*	139

Values are means ± S.E.M. n = 5 animals/group. Negative control was treated with the vehicle used to dilute the drug (DMSO 5 %). 5-Fluorouracil was used as positive control. *$p < 0.05$ compared with the negative control by ANOVA followed by Newman-Keuls test.

Table 3

Antiproliferative potentiality of arylacetamides **1-10** on human tumor lines and primary culture of peripheral blood mononuclear cells (PBMC) and analysis of hemolytic capacity.



d	R	IC$_{50}$ [µg/mL (µM)]						H
		HL-60	**OVCAR-8**	**SF-295**	**HCT-116**	**HepG-2**	**PBMC**	
	H	6.0 (35.38)	8.5 (50.1)	> 10 (>	8.4 (49.53)	4.5 (26.7)	7.6 (44.6)	0
phenylacetamide		5.4-6.8	7.5-9.7	59.0)	7.5-9.4	3.8-5.4	6.1-9.4	
	Cl	1.0 (4.9)	> 10 (> 49.0)	5.6 (27.44)	3.4 (16.66)	2.4 (11.8)	3.4 (11.8)	5
lorophenyl)acetamide		0.6-1.5		4.1-7.7	2.5-4.5	2.0-2.9	3.0-3.8	
	Br	8.4 (33.8)	7.4 (29.78)	9.8 (39.44)	7.0 (28.17)	4.2 (16.7)	5.7 (22.9)	0
yl)-2-chloroacetamide		6.7-10.5	6.4-8.6	8.2-11.8	5.5-8.9	3.2-5.4	4.7-6.8	
	NO_2	4.8 (22.37)	4.4 (20.5)	8.9 (41.47)	4.7 (21.9)	2.4 (11.2)	3.0 (14.1)	0
itrophenyl)acetamide		4.2-5.9	4.1-4.8	7.7-10.3	3.8-5.8	1.8-3.2	2.3-4.0	
	CH_3	5.9 (32.13)	6.9 (37.58)	> 10 (>	10.0 (54.46)	2.8 (15.3)	> 25 (>	0
p-tolyl)acetamide		4.6-7.7	6.0-8.1	54.5)	8.6-11.5	2.3-3.5	136.1)	
	OCH_3	> 10 (>	> 10 (> 50.1)	> 10 (>	> 10 (> 50.1)	nd	nd	
thoxyphenyl)acetamide		50.1)		50.1)				
	$COCH_3$	8.3 (39.23)	> 10 (> 47.3)	> 10 (>	9.6 (45.36)	1.9 (9.0)	5.2 (24.4)	0
yl)-2-chloroacetamide								

	7.3-9.5		47.3)	7.8-11.9	1.5-2.4	4.1-6.6
SO_2NH_2	5.6 (22.52)	9.8 (39.41)	> 10 (>	6.5 (26.14)	2.4 (9.6)	6.1 (24.5)
amoylphenyl)acetamide	4.6-7.0	6.8-14.2	40.2)	4.5-9.1	1.8-3.1	4.9-7.7
COOH	> 10 (>	> 10 (> 46.8)	> 10 (>	> 10 (> 46.8)	nd	nd
tamido)benzoic acid	46.8)		46.8)			
$COOCH_2C_3$	> 10 (>	7.5 (31.03)	> 10 (>	> 10 (> 41.4)	2.9 (12.0)	4.8 (19.8)
oacetamido)benzoate	41.4)	6.3-8.9	41.4)		2.3-3.7	3.6-6.3
n -	0.02 (0.04)	1.3 (2.4)	0.2 (0.4)	0.01 (0.02)	0.11 (0.21)	1.9 (3.2)
	0.01-0.02	1.0-1.9	0.2-0.3	0.01-0.02	0.17-0.25	1.4-2.4

Data are presented as IC_{50} values and 95% confidence intervals for leukemia (HL-60), ovarian (OVCAR-8-1), glioblastoma (SF-295), colon (HCT-116), and liver (HEPG-2) tumor lines determined by MTT assay, and for primary culture of human peripheral blood mononuclear cells (PBMC) performed by Alamar Blue assay. Doxorubicin was used as positive control. All experiments were performed in duplicate and represented independent biological evaluations. Nd: not determined. *Values are presented as percentage of hemolysis ± S.E.M. at 250 µg/mL. ***p* < 0.05 compared with the negative control by ANOVA followed by Newman-Keuls test.

Table 4

Physicochemical properties of compounds (**1-10**).

Compound	R	σ_R[a]	ClogP[b]	vdWV[c] (Å^3)	Dipole moment[d] (D)
1	H	0	1.75	143.68	0.957
2	Cl	0.23	2.35	157.61	2.580
3	Br	0.23	2.52	161.93	2.287
4	NO_2	0.78	1.69	166.65	5.880
5	CH_3	-0.17	2.26	160.45	0.977
6	OCH_3	-0.27	1.59	169.78	2.148
7	$COCH_3$	0.50	1.31	179.88	4.063
8	SO_2NH_2	0.57	0.35	191.47	6.352
9	COOH	0.45	-1.66	171.45	2.484
10	$COOCH_2CH_3$	0.45	2.11	205.83	3.078

[a]σ_R: Hammet's substituent electronic constant; obtained from Hansch and Leo (1979); [b]ClogP: calculated partition coefficient; calculated using MarvinSketch 6.2.0 [ChemAxon, 2012, (https://www.chemaxon.com/marvin/sketch/index.php)]; [c]vdWV: van der Waals volume; calculated using MarvinSketch 6.2.0; [d]calculated using HyperChem [HyperChem(TM) Professional 8.0.8, Hypercube, Inc., 1115 NW 4th Street, Gainesville, Florida 32601, USA].

Table 5

Effects of synthetic arylacetamides on relative weight of key organs and on tumor growth in **CB-17 mice** bearing HCT-116 colon carcinoma after 15 days of intraperitoneal treatment.

Group	Dose (mg/kg /day)	Mice weight (g)	Survival	Liver (g/100g)	Kidney (g/100g)	Lungs (g/100g)	Heart (g/100g)	Tumor (g)	Tumor inhibition (%)
Negative control	-	20.03 ± 0.43	12/12	4.95 ± 0.14	1.46 ± 0.04	0.67 ± 0.04	0.51 ± 0.02	0.73 ± 0.04	-
5-Fluorouracil	15	18.53 ± 0.88	7/12	6.18 ± 0.39*	1.59 ± 0.10	0.82 ± 0.05	0.59 ± 0.05	0.32 ± 0.06*	56.6*
Compound 2	25	19.00 ± 0.31	12/12	4.99 ± 0.23	1.45 ± 0.06	0.72 ± 0.04	0.61 ± 0.04	0.57 ± 0.02*	21.2*
Compound 3	25	18.60 ± 0.44	12/12	5.08 ± 0.26	1.54 ± 0.06	0.78 ± 0.04	0.56 ± 0.02	0.53 ± 0.04*	27.5*
Compound 4	25	19.98 ± 0.70	12/12	5.02 ± 0.31	1.33 ± 0.08	0.71 ± 0.04	0.51 ± 0.03	0.67 ± 0.05	8.2

Values are means ± S.E.M. n=12 animals/group. Negative control was treated with the vehicle used to dilute the drug (DMSO 5 %). 5-Fluorouracil was used as positive control. *$p < 0.05$ compared with the negative control by ANOVA followed by Newman-Keuls test.

Table 6

Hematological profile of **CB17 mice** bearing HCT-116 colon carcinoma after 15 days of intraperitoneal treatment with synthetic arylacetamides.

Group	Dose (mg/kg /day)	Erythrocytes (10^6 /mm^3)	Hemoglobin (g/dL)	Hematocrit (%)	Platelets (10^3 /mm^3)	Total leukocytes (10^3 /mm^3)	Differential counting of leukocytes (%)		
							Lymphocytes	Monocytes	Granulocytes
Negative control	-	9.88 ± 0.32	12.59 ± 0.47	46.31 ± 1.58	633.6 ± 56.20	2.62 ± 0.41	42.17 ± 3.65	29.72 ± 3.65	28.20 ± 4.31
5-Fluorouracil	25	8.27 ± 0.20*	10.81 ± 0.41*	41.03 ± 1.81*	653.3 ± 102.5	1.53 ± 0.15*	41.08 ± 6.36	31.83 ± 3.28	27.08 ± 3.57
Compound 2	25	9.81 ± 0.34	13.19 ± 0.29	50.14 ± 1.10	549.6 ± 51.20	3.23 ± 0.11	27.16 ± 1.66	40.72 ± 1.01	31.04 ± 1.97
Compound 3	25	9.77 ± 0.20	12.77 ± 0.09	48.46 ± 0.61	453.6 ± 71.46	3.33 ± 0.61	37.90 ± 4.61	36.70 ± 2.96	25.34 ± 3.56
Compound 4	25	8.98 ± 0.07	12.26 ± 0.23	46.40 ± 1.11	473.3 ± 56.21	1.86 ± 0.23	40.30 ± 4.42	31.72 ± 3.14	27.96 ± 2.16

Values are means ± S.E.M. n = 12 animals/group. Negative control was treated with the vehicle used to dilute the drug (DMSO 5 %). 5-Fluorouracil was used as positive control. *p < 0.05 compared with the negative control by ANOVA followed by Newman-Keuls test.